Recent Omics Advances in Hair Aging Biology and Hair Biomarkers Analysis

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Abstract

Aging is a complex natural process that leads to a decline in physiological functions, which is visible in signs such as hair graying, thinning, and loss. Although hair graying is characterized by a loss of pigment in the hair shaft, the underlying mechanism of age-associated hair graying is not fully understood. Hair graying and loss can have a significant impact on an individual's self-esteem and self-confidence, potentially leading to mental health problems such as depression and anxiety. Omics technologies, which have applications beyond clinical medicine, have led to the discovery of candidate hair biomarkers and may provide insight into the complex biology of hair aging and identify targets for effective therapies. This review provides an up-to-date overview of recent omics discoveries, including age-associated alterations of proteins and metabolites in the hair shaft and follicle, and highlights the significance of hair aging and graying biomarker discoveries. The decline in hair follicle stem cell activity with aging decreased the regeneration capacity of hair follicles. Cellular senescence, oxidative damage and altered extracellular matrix of hair follicle constituents characterized hair follicle and hair shaft aging and graying. The review attempts to correlate the impact of endogenous and exogenous factors on hair aging. We close by discussing the main challenges and limitations of the field, defining major open questions and offering an outook for future research.

Keywords: Hair, aging, hair follicle, hair graying, oxidative stress

1. INTRODUCTION

Aging is a universal, natural, multifactorial physiological process characterized by time-dependent alterations in biological, physiological, and neurological processes which lead to progressive functional decline. Numerous aging theories and hypotheses have been proposed to describe aging (Jin, 2010; López-Otín et al., 2013; Yang et al., 2023). Hair thinning, loss, and graying are undesirable consequences of the aging process and are accelerated by intrinsic genomic stability and extrinsic factors (Matsumura et al., 2016; O'Sullivan et al., 2021). However, the mechanism is still unclear. Our pre-historic ancestors were much hairier than we are today. However, the rationale for the diminished hairiness of modern-day individuals is unknown. Gray hair is a visual marker of aging, but whether aging is the ultimate risk factor for gray hair remains debatable, and there is a need for a better understanding of the mechanisms that drive hair aging.

In modern life, physical appearance remains critical, where wrinkles, gray hair, hair thinning, and loss are visible signs of human aging. The scalp hair remains extremely important to an individual's self-esteem and self-confidence, and their loss and or graying substantially impact the quality of life, leading to depression, anxiety, and other serious mental health complications. To overcome these issues, consumers constantly strive for hair-care products and demand quick and easy solutions. The hair care industries are constantly exploring pharmacological targets and active products to meet such consumer needs, including the desire to have beautiful, healthy, and young-looking hair. With the increasing demand for hair products, the global haircare market is expected to reach USD 211.1 billion by 2025 (GVR, 2018). Numerous products on the market help to fight the signs of aging, but all of them treat the symptom and not the cause. Hence, to find the main cause, it is crucial to understand the complex biology of hair aging, to describe the age-dependent physiological changes that occur in the hair shaft (HS) and hair follicle (HF), and to identify hair aging biomarkers.

Various extrinsic factors (ultraviolet radiation, air pollution, smoking, nutrition, and lifestyle) and intrinsic factors related to individual genetic, epigenetic, and endogenous oxidative stress impact HF (Trüeb, 2021). Therefore, this model has the potential to provide an in-depth understanding of biological aging, which remains a consequence of two independent biological processes, one programmed loss of functionality and other damage-related changes (Jin, 2010). However, Sinclaire and team (Yang et al., 2023) proposed that aging is the result of losing critical epigenetic information or instructions that cells need to continue functioning. According to this "Information Theory of Aging", the underlying cause of mammalian aging is loss of information in cells, and not just accumulation of damage. In case of hair aging, age-independent hair graying has been linked with age-associated pathologies, including Alzheimer's disease (Mendelsohn and Larrick, 2020), Parkinson's disease (Jucevičiūtė et al., 2019), and cardiovascular disease (ElFaramawy et al., 2018). Thus, a unique structure, multicellular interaction, the cyclic activity of growth, sensitivity to intrinsic and extrinsic factors, and association with age-associated disorders make hair aging a unique model for studying biological aging.

Molecular methodologies have been embraced by those attempting to understand the genetic makeup and biological aspects of hair aging (Pavan and Sturm, 2019; Williams et al., 2021). Substantial efforts were undertaken to understand the cause of age-associated hair thinning, loss, and graying (O'Sullivan et al., 2021; Tobin, 2015; Williams et al., 2021). However, it is still poorly understood and seems to be extremely complicated. Due to the potential of advanced "omics" to accommodate multi-parameter measurement, these technologies are being widely used in the discovery of clinical disease biomarkers, unfolding complex pathologies, aging, and age-associated disorders (Adav et al., 2016; Adav et al., 2019; Adav and Sze, 2020; Adav and Wang, 2021; López-Otín et al., 2013). An integrated omics platform, including genomics, proteomics, transcriptomics, metabolomics, and bioinformatics,

with morphology, including ultrastructural morphology and quantitative immunohistomorphometry could provide in-depth insight into age-associated alterations in the functional network of genes, proteins, and metabolites. Omics platforms have the potential to reveal the complex interactions of different hair keratins; divulge age-dependent hair protein abundances, age-dependent hair pigmentation, and causes of hair loss; translate discoveries into new clinical aging biomarkers; and help develop effective hair therapies.

The main objectives of this review were to provide recent omics advances in hair aging, including age-associated HS protein abundances, hair thinning, loss, and graying. Given the complexity of hair proteome and metabolome and their dynamic nature, we highlighted recent biomarker discoveries in hair aging. We attempted to correlate the impact of endogenous and exogenous factors with hair aging and discussed the main challenges and limitations in the field.

2. HAIR SHAFT

2.1 Hair shaft- a precious bio-sample in research

The hair, an evolutionarily conserved structure from hair follicles, mainly consists of proteins. The hair follicle is surrounded by a rich capillary system providing the necessary nutrients for growing hair. In other words, the blood components interact with hair molecules during the growth of the HS, and their composition is known to represent the blood composition to a certain extent (Kempson and Lombi, 2011). Therefore, hair samples can be vital in deciphering the aging process, recording environmental exposure, and providing evidence in solving mysteries in forensic sciences. Further advantages of the hair samples over blood, urine, and tissue remain their solid and durable nature, substantially longer detection window (months to years), and less invasiveness.

The multicellular interaction system of the epithelium, mesenchyme, and neuroectoderm, which undergoes a unique cyclical activity of growth (anagen), regression (catagen), rest (telogen), shedding (exogen), and regrowth, makes the hair follicle a highly comprehensible model with exclusive opportunities for studying age-related changes. Being mini organs of the skin, HFs express a set of heterogeneous clock genes that can modulate the central circadian system and they have their own stem-cell system to sustain cellular and tissue turnover. Life style, particularly sleep quality is thought to be associated with hair loss(Agaoglu et al., 2021). Sleep and wake cycles are intertwined with the circadian clock and growing evidence indicates an association between the circadian clock and the aging process. Circadian genes play a key role in the regulation of the hair cycle, hair aging and pigment production (Liu et al., 2023). Since these genes are synchronized with the central clock while telomere and telomerase activity have been linked with the circadian system and aging, HFs may assist in understanding the human biological clock. During evaluation of the fate of HF stem cells (HFSCs) during aging, Matsumura et al (Matsumura et al., 2016) concluded that aging is primed by DNA damage, which further induced depletion of type XVII collagen (COL17A1) and triggered cell differentiation leading to HF miniaturization. Furthermore, these authors established a link between intrinsic genomic instability in stem cells and epithelial tissue aging.

Recently, several researchers (Chu et al., 2020; Karim et al., 2021; Parker et al., 2016; Plott et al., 2020) have exploited the application of HS protein profiles and genetically variant peptides (GVPs) in discriminating individuals as well as to determine their demographics including age, gender, and ethnic group. Hair has been used as a valuable specimen sample to investigate retrospective xenobiotic exposure and to monitor the intake of such lipophilic compounds (Jang et al., 2019) including Δ^9-tetrahydrocannabinol, cocaine, opiates and amphetamines, heavy metal lead, and therapeutic drugs like chloroquine, cisplatin, mephenesin and p-aminobenzoic acid can all be incorporated into the HS (Carré et al., 2020). The

156 importance of hair samples in the era of precision medicine (Ademola Adeola et al., 2018), and
157 forensics (Duong et al., 2021) have been reviewed, while recent applications have been
158 tabulated in Table 1.

159 Hair samples have been used in the omics-based evaluation of virological response to
160 atazanavir and lopinavir drug treatment in HIV-infected adults (Gandhi et al., 2009), isoniazid
161 levels in tuberculosis patients (Gerona et al., 2016), estimating chronic psychosocial stress
162 (Wright et al., 2015), monitoring alcohol abuse through metabolites (Crunelle et al., 2014),
163 differentiating normoglycemic and hyperglycemic in diabetes mellitus (Kobayashi and Igimi,
164 1996), drug abuse (Pichini et al., 2014), cancer (Mistry et al., 2012), and many more. Hair
165 keratins are crucial biomaterials for diverse regenerative applications, including controlled
166 drug release, tissue engineering, and fabrication of films, sponges, and hydrogels (Chua et al.,
167 2020; Gentile et al., 2021; Lai et al., 2021; Vasconcelos and Cavaco-Paulo, 2013; Wang et al.,
168 2015). In summary, hair is a valuable biomaterial and a precious biological sample in aging
169 studies and clinical and forensic science.

170 **2.2 Omics analysis of hair shaft and discovery of hair aging biomarkers**

171 Omics technologies have far-reaching applications beyond clinical medicine and are
172 considered a novel approach to evaluating the genetic basis of disease, determining the function
173 of proteins in health and disease, understanding biological processes, and much more. The
174 analytical cost might have limited the development of proteomics, but its applications, similar
175 to genomics, are promising. The advantage of proteomics is that it provides better proteome
176 dynamics over the genome. It has also been embraced in evaluating cosmetic treatment-induced
177 hair damage (Sinclair et al., 2012) and monitoring cocaine in a single strand of hair sample for
178 forensic purposes (Porta et al., 2011). The various mass spectrometric platforms, including gas
179 chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry
180 (LC-MS), matrix-assisted laser desorption/ionization (MALDI), and desorption electrospray

ionization (DESI) mass spectrometry imaging are being used in hair proteome profiling (Adav et al., 2018; Barthélemy et al., 2012; Franklin et al., 2020; Hsia et al., 2011; Subbaiah et al., 2018). Despite the significant importance of scalp hair and technological advances in mass spectrometry, limited literature exists on hair proteomics.

2.2.1 Protein biomarkers of hair shaft aging

HS comprises the cuticle, cortex, and medulla. An outer protective layer of cuticle cells comprises cuticular proteins and high sulfur keratin-associated proteins (KAPs); lipids surround the cortex, which in turn surrounds an intermittent medulla column at the center of the shaft (Lee et al., 2006). The cortex contains 500–800 keratin intermediate filaments (KIFs) (Dawber, 1996) that are categorized into two groups (i) the acidic type I keratins (KRT31–38, containing 9 members) and (ii) the neutral basic type II keratins (KRT81–86, containing 6 members) (Langbein et al., 2001). The complex interaction between type I and type II keratins leads to the formation of heteropolymers (Parry et al., 2007). The expression of KIFs and KAPs has been well established (Rogers et al., 2008), but their exact functions, role in maintaining hair quality, and alterations with age remain unclear. Alterations in the hair structure, characteristics, extrinsic damage, and responses to extrinsic damage in different races and ethnic groups with aging are reviewed elsewhere (Maymone et al., 2021) and not covered herein.

The proteomics approach allows the identification and comparison of abundances of thousands of proteins in a single experiment. It provides dynamics of protein expression, posttranslational modifications (PTMs), regulation, interactions, and function (Diz et al., 2012). The studies on the transcriptome or the genome are considered inadequate in understanding the phenotype because of the lack of convergence between the proteome and the transcriptome (Schwanhäusser et al., 2011). Using state-of-the-art proteomics technologies,

about 300 proteins, including keratin, KAPs, and KIFs, were identified in the HS (Adav et al., 2018; Barthélemy et al., 2012; Lee et al., 2006).

To study the impact of aging on the HS, Plott et al., (Plott et al., 2020) analyzed hair samples from single individuals with 44 years gap and noted 2-fold changes in the abundance of 54 proteins, including the proteins of the cuticle (KRT40, 82, KRTAP16-1, S100A3). These authors noted the decreased abundance of cuticular proteins with increasing age, which can be interpreted as a loss of cuticle with age, as depicted in other studies (Thibaut et al., 2010). To understand the natural aging of human hair, Thaibaut et al (Thibaut et al., 2010) analyzed a very long scalp HS (length > 2.4 m) from a woman who had not cut her hair for 26 years and observed hair diameter ranging from medium to thin at the 'root' (~ 65-70 μm) and from medium to coarse at the tip (~65-95 μm). The physical findings of loss of cuticle cells 1 m from the root to the tip end were correlated with a progressive and linear decrease in the total amount of cuticle-bound 18-MEA from root to about 1.0-1.25 m hair length, leading to the conclusion that hair diameter decreased with age. In parallel, Robbins (Robbins et al., 2012) evaluated a total of 1414 women (age 18-66 years) hair samples and observed hair diameter to increase from ages 20 to 40 years, and decreased subsequently. In addition, phototrichogram studies in 1357 subjects (674 males and 683 females between the ages of 10 and 69) by Kim et al (Kim et al., 2013a) and 45 subjects (age 17-73 years) by Lee et al (Lee et al., 2012) confirmed that hair diameter reduced with chronological aging. The decreased expression of these cuticular proteins can be seen as a high risk to the cortex and, thus, the HS structure. Other studies also documented the altered HS diameter with age, particularly decreasing with an increase in age (Kim et al., 2013b; Robbins et al., 2012). The accumulated literature reveals the loss of cuticular proteins and decreased HS diameter with age, but it remains debatable and challenging to conclude whether it is an aging impact or wear and tear due to environmental factors; hence the need for well-designed studies.

Laatsch et al., (Laatsch et al., 2014) isolated cuticle cells and analyzed them by shotgun proteomics, revealing that the cuticle has a distinctly different protein profile from those of the total hair. These authors found proteins KRT32, 40, and 82 to be more abundant in the cuticle, while proteins KRT31, 33A, 33B, 38, 39, 83, 85, and 86 were less abundant. While the authors used core proteomics, further validation of KRT32, 40, and 82 abundances with an orthogonal validation tool like Western blotting will strengthen the conclusion. Including more replicates, especially biological replicates, as this study by Laatsch et al. (5-6 replicates) showed, provide better statistical confidence since they help to minimize random noise. The histological studies of hair cuticles by transmission electron microscopy (TEM), scanning electron microscope (SEM), X-ray photoelectron spectroscopy (XPS), secondary ion mass spectroscopy (SIMS), and atomic force microscopy (AFM) in conjunction with protein analytical techniques revealed three layers in cuticles including A-layer, exocuticle, and endocuticle (Rogers, 2019). An age-associated abundance of these cuticle proteins and their localization may assist in understanding the cuticular structure of hair and the complex role of these proteins in hair structure stability and aging. The content of mRNAs encoding hair keratins KRT33A and 34 and KAPs declined with age (Giesen et al., 2011). Of the studied 39 KAP genes, seven members of the family, including the KAP4 family, were significantly downregulated in aged hair (Giesen et al., 2011). It is important to note that KAP4 represents the largest KAP family presumed to be localized in the middle and upper cortex and involved in the terminal keratinization of the cortex (Kariya et al., 2005). With increasing age, the decline in KAP4 gene expression can be interpreted as a decrease in HS stability and flexibility.

During a long anagen phase (2–6 years), hair grows at a rate ranging from 0.8 to 1.3 cm\month depending on the ethnic origin and the individual parameters (Loussouarn et al., 2005) and attains hair length from 30 to 100 cm in Asian. The long hairs represent an accessible model to describe hair aging. Thibaut et al.(Thibaut et al., 2010) chose a very long scalp HS

(length > 2.4 m) from a woman who had never cut her hair for 26 years, divided it into 60 cm segments and profiled each segment's protein expression using two-dimensional gel electrophoresis. The results revealed structural protein differences at the root, middle, and tip of the HS fiber and a loss of some 3–30 kDa KAPs from the tip part of the fiber, indicating a loss of integrity at the tip region. Although some support comes from AFM and physical data presented by the authors, analyzing such segments with an advanced high-throughput LC-MS/MS could provide more in-depth knowledge.

2.2.2 Post-translational modifications of the hair shaft proteins and their biomarker potential

With technological advances, aging research has shifted its focus to a healthy lifespan. Cosmetic industries and biologists are putting effort into understanding the hair aging process to modulate (delay or minimize or reverse the effect) hair aging. PTMs have been linked to aging (Cloos and Christgau, 2004; Robinson and Robinson, 2001). PTMs are mainly classified into two categories, i.e., enzymatic and nonenzymatic alteration of specific groups to amino acid side chains, and both PTMs can undergo age-related alterations (Adav and Sze, 2020). While profiling HS proteome, Lee et al. (Lee et al., 2006) noted novel modifications like methylation, dimethylation, and trimethylation in the keratins, while Adav et al. (Adav et al., 2018) discovered deamidated type I keratin proteins KRT33A, 33B, 34, and 35; type II such KRT82-86; and several KAPs.

Methylation is a well-known PTM mediated by enzyme methyltransferases, where methyl groups are added to nitrogen or oxygen (N- and O-methylation, respectively) on amino acid side chains. It increases protein hydrophobicity or neutralizes a negative charge when bound to carboxylic acids (Marmorstein, 2003). Protein methylation plays a key role in cellular and biological processes and has been implicated in signal transduction, gene transcription,

DNA repair, and mRNA splicing (Yang and Bedford, 2013). The roles of DNA methylation in aging, rejuvenation, and age-related diseases have been well established (Cruz et al., 2018; Field et al., 2018; Johnson et al., 2012). The methylation clock correlates cross-sectionally with apparent biological age and is predictive of future aging phenotypes, such as cancer, cardiovascular diseases, and cause of mortality (Ambatipudi et al., 2017; Fernandes Durso et al., 2017). Although methylation/dimethylation/trimethylation of hair proteins is documented, site-specific methylation and its role in hair aging are yet to be established. Naue et al. (Naue et al., 2021) attempted to establish age-dependent DNA methylation (DNAm) in plucked hair samples. This study determined DNA methylation patterns of 10 loci, and the correlation between DNAm and age for ELOVL2, KLF14, RPA2, TRIM59, and ZYG11A was observed. However, more efforts are still required to establish a suitable protocol to achieve sufficient high-quality DNA for a DNAm analysis from shed hair in contrast to plucked hair.

Deamidation of the protein residues asparagines (Asn) and glutamine (Gln) can occur spontaneously, and it may alter protein structure, function, and stability over time (Kato et al., 2020). Lifelong proteins like elastin in the lung (Shapiro et al., 1991), crystallins in the lens (Lynnerup et al., 2008), and dentin in teeth (Spalding et al., 2005) were mostly targeted to establish a correlation between deamidation and age. The deamidation pattern detected in normal eye lens protein (crystallin) indicated that aging is a major contributor to deamidation (Hains and Truscott, 2010). These authors found increased levels of deamidation at sites αA, Q147; αB, N78, Q108; βA4, Q64, N82; βB1, N67, N69; γC, N24; γD, N160; γS, N14, N16, N76. The impact of site-specific deamidation of hair proteins on their structure and function has not been proven, but the protein deamidation was proposed to represent a “molecular clock” and is linked to tissue aging and regulating biomolecule longevity (Adav et al., 2018; Hao et al., 2017; Lindner and Helliger, 2001; Robinson and Robinson, 2001). Plott et al. (Plott et al., 2020) noted higher deamidation in the HS samples stored for at least 10 years. Thus,

deamidated hair proteins could be a key aging biomarker. However, the quantitative correlation between the extent of deamidation and hair age needs to be established. Briefly, PTMs of keratins, including deamidation, methylation, glycosylation, and citrullination could act as a aging biomarker, but more detailed research is required to establish the corelation and validate them.

Glycosylation plays an important role in the structure and function of many proteins, including hair proteins. Protein glycosylation is an enzyme-catalyzed process that attaches glycans to proteins, lipids and other organic molecules in a site-specific manner. Based on the atoms to which glycans are linked, glycosylation is classified as N-linked, O-linked and C-linked glycosylation., respectively. This protein modification has significant implications on protein folding, conformation, protein targeting, cell-to-cell communication, cell-matrix interaction, protein stability, and various molecular recognition processes such as viral or bacterial infection, cancer and aging (Banerjee et al., 2017; Breloy and Hanisch, 2018). The presence of N-glycans lacking terminal galactose residues linked to Asn297 of IgG heavy chains (IgG-G0) is considered a marker of biological aging. The first indication of an increased expression of IgG-G0 in aging came from a pioneering study published in 1988 (Parekh et al., 1988). Since then, age-associated accumulation of IgG-G0 has been observed by several other researchers and it has inspired the development of high throughput approaches for the detailed analysis of a very large number of plasma or serum specimens of various cohorts (Adav et al., 2015; Memarian et al., 2021). Altered glycoproteome of plasma and other tissues from the brain, heart, liver etc., and their roles in different diseases and aging processes have been well established (Franzka et al., 2021; Medzihradszky et al., 2015; Sato and Endo, 2010; Vanhooren et al., 2010). Paton et al (Paton et al., 2021) reviewed altered protein glycosylation profiles that are associated with aging and age-related diseases.

Human hair proteins may undergo various modifications including glycosylation during synthesis and maturation. Glycosylation of proteins has been found to contribute to their structural integrity, hydration, and other functional properties. Proteoglycan and associated glycosaminoglycan expression patterns in the human hair follicle have been evaluated by immunohistochemistry and immunohistofluorescence techniques (Malgouries et al., 2008) and it was noted that the dermal part of the hair follicle contained high amounts of extracellular proteoglycans such as perlecan, versican, aggrecan, biglycan and their saccharidic moieties. The glycosylation patterns of hair shaft proteins may vary with age and health conditions. Very few studies have been conducted on hair shaft protein glycosylation. These studies are needed to fully understand the extent of glycosylation, its functional implications, and its potential applications in the field of hair science and hair aging. Understanding the glycosylation patterns and their influence on hair structure and properties may also provide insights into the development of improved hair care and cosmetic products.

Non-enzymatic post-translational protein modifications, also referred to as degenerative protein modification, include glycation, advanced glycation endproducts (AGEs), oxidation, carbonylation, carbamylation, etc. These impart deleterious structural and functional changes in proteins and impair their normal function (Adav and Sze, 2020). Protein glycation is a non-enzymatic reaction between a reducing sugar and the free amino group of a target protein. Glycated proteins are formed through a two-step nonenzymatic reaction called the Maillard reaction, in which the free amino group of a protein and the aldehyde group of a glucose form a Schiff base or aldimine, and in the second step, aldimine undergoes a slow and almost irreversible Amadori rearrangement to become a stable ketoamine or Amadori product that is a glycated protein.

The formation and accumulation of AGEs alter the structure and function of proteins, develop many age-associated morbidities and are an inevitable component of the aging process (Chaudhuri et al., 2018). AGE composition of hair and nails (Kishabongo et al., 2014) has also been studied as an index for diagnosis of diabetes mellitus, with results confirming that AGEs levels in hair could indeed correlate positively with serum HbA1C levels and breaking of hair (Shomode et al., 2014). However, such studies in hair and the correlation of AGEs with age are still lacking.

2.2.3 Hair metabolites as biomarkers of hair aging

The large-scale, unbiased profiling of metabolites has succeeded in the post-genomic era of biology. Unlike genomics, transcriptomics, and proteomics, metabolomics reflects the phenotype of living organisms, understanding biological mechanisms, provides quantitative metabolites information, and assists in discovering biomarkers (Bujak et al., 2015; Mastrangelo and Barbas, 2017). Metabolomics has been well-adopted in various cancers, including lung, colorectal, bladder, breast, gastric, oesophageal, and thyroid (Armitage and Ciborowski, 2017; Beger, 2013). Metabolomic signatures of aging have been recently reviewed (Adav and Wang, 2021). To understand hair aging better, Waki et al. (Waki et al., 2011) analyzed hair from 20 ± 5 years (younger) and 50 ± 5 years (older) volunteers using the matrix-free laser desorption/ionization IMS technique. The mean signal intensities at m/z 153.00 (speculated to be dihydrouracil) and 207.04 (putatively 3,4-dihydroxymandelic acid (DHMA) in the cortex) were higher in the 20 years old group than in the 50 years old group, while the ion at m/z 164.00 (*O*-Phosphoethanolamine) had significantly higher intensity in the 50-year-old group. According to Le Stunff et al. (Le Stunff et al., 2002), O-phosphoethanolamine is a sphingosine-1-phosphate (S1P) metabolite. The aging process leads to increased secretion of S1P into the circulation, and this increased S1P might be processed to O-phosphoethanolamine and 2-hexadecanal by S1P-lyase following internalization by the cells forming the hair structure

(Schwab et al., 2005). Although these three molecules (i.e., m/z 153.00 (Dihydrouracil), 207.04 (DHMA), and 164.00 (*O*-Phosphoethanolamine)) have been proposed as hair aging biomarkers, further confirmed identification and quantitative validation are very much essential. If the validated quantitative correlation between their content and age is established, then it has significant importance in forensic applications since age is a crucial information in an investigation.

Along the length of the shaft, significant cuticle damage was accompanied by ceramides and 18-Methyl Eicosanoic Acid (18-MEA) decline, as well as a progressive decrease in keratin-associated protein content (Thibaut et al., 2010). 18-MEA is a branched-chain fatty acid, covalently bound, possibly via thioester or ester linkage, to the cuticle surface of hair fibers (Tokunaga et al., 2019). This protein-fatty acid bonding offers a hydrophobic surface characteristic and protects hair cuticles by reducing friction between hair fibers. Through the redox lipidomic approach, Cornellison et al. (Cornellison et al., 2011) characterized and profiled selected lipids in hair and found that cholesterol and cholesterol derivatives are highly susceptible to oxidative damage. The systematic quantitative age-dependent characterization of oxidative products and its correlation with the extent of hair damage might add to the knowledge of hair aging.

Lipids play a key role in protecting the HS from environmental as well as chemical damage, prevention of hair breakage and thinning, serving as a barrier against moisture loss and improving glossiness of the HS. The lipid composition of human hair is systematically reviewed by Csuka et al (Csuka et al., 2023). Based on origin i.e. sebaceous glands or hair matrix cells, hair lipids are classified as exogenous or endogenous. Exogenous hair lipids include free fatty acids (FFAs), triglycerides, cholesterol (CH), wax esters and squalene (SQ) while endogenous lipids are FFAs, CH, ceramides (CERs), glycosylceramides, cholesterol sulfate (CS) and 18-methyleicosanoic acid (18-MEA) (Coderch et al., 2017). Treatments such

as bleaching, dyeing, perming, straightening, surfactant use, UV radiation exposure as well as aging and graying, result in the reduction of lipids such as C22:1FA (erucic acid), C22FA (behenic acid), C24FA (lignoceric acid), C24:1FA (nervonic acid), C26FA (cerotic acid) and 18-MEA, in proportion to the severity of hair damage (Joo et al., 2016; Tokunaga et al., 2019). Lipid removal reduces the tensile strength, glossiness, and fineness of hair, while increasing permeability and desorption (Bildstein et al., 2020). Gray hair is a hallmark of aging and their lipid profile showed lower levels of 18-MEA and decreased de novo synthesis of dihydroceramide (Lee et al., 2014). Similarly, while investigating the role of lipids in the process of hair aging from brown to gray, Coderch et al (Coderch et al., 2022) found a loss of lipids, primarily FFA and polar lipids in the brown-to-gray transition. Lipids in the HF root differ between brown and gray hair, particularly, phospholipids, vitamin D3 and cholesterol were significantly low in gray hair follicles (Wang et al., 2020). Hair lipid content decreased with age in women (Trüeb et al., 2018b). On the contrary, Brosche et al (Brosche et al., 2001) noted an increase in hair cholesterol in women as they age, but not in men. With advancing age, reduced lipid content of the cuticle, cortex, and medulla was observed (BioMed research internationalOliver et al., 2019). Importantly, hair lipid content and its composition differ significantly with ethnicity e.g. African hair has, in general, higher lipid content and specifically cholesterol ester and cholesterol sulphate (Cruz et al., 2013). Ji et al (Ji et al., 2013) noted higher quantities of integral lipids, fatty acids, cholesterol, and wax esters in Asians when compared with Caucasians and African-Americans.

The proteome and metabolome are dynamic and vary with time, diet, exercise, immune status, sleep pattern, exposure to environmental factors, constant washing, cosmetic treatment, and drugs (Miolo et al., 2020). With hair growth, endogenous metabolites are constantly incorporated into the growing HS via blood and sebum. Hair has the potential not only to retain information about long-term environmental exposure and drugs intake but can also reveal a

person's age, sex, smoking habit etc., which makes the hair a precious diagnostic tool in forensic science to obtain an individual's lifestyle (Chojnacka et al., 2006; Sauvé et al., 2007). The incorporation depends on the physiochemical properties of the metabolites, including their molecular weight, lipophilicity, acidic\basic nature, pKa values, melanin content, etc. (Pragst and Balikova, 2006a). The intracellular pH of keratinocytes remains acidic, while the pH of the melanocytes remains between 3.0 and 5.0 (Pötsch et al., 1997). At the lower pH, melanin affinity for basic metabolites\drugs leads to the accumulation of lipophilic and basic drugs (Claffey et al., 2001). The HS is stable and can potentially retain endogenous compounds for several months (Eisenbeiss et al., 2020b; Sulek et al., 2014). In short, proof-of-concept metabolomics of the HS studies correlating to drug intake has been documented (Eisenbeiss et al., 2020a; Scott and Nakahara, 2003). However, more efforts are required to adopt hair metabolomics in forensic sciences. Further, a very limited metabolomic approach has been applied in hair aging studies, possibly due to the metabolomics database's limitation.

2.3 Omics of hair shaft graying

Hair graying is universal and is one of the earliest visible indicators of human biological aging, which remains an undesired consequence of the aging process and works against most people's desire to look younger than their age. It occurs in all individuals with varying degrees, but onset varies between individuals, ethnicities, and geographical locations (O'Sullivan et al., 2021). According to the 50-50-50 rule of thumb (derived from a single ethnic population, i.e., Australian subjects), 50% of the population has about 50% gray hair at the age of 50 years (Keogh and Walsh, 1965), which has been revisited by Panhard et al. (Panhard et al., 2012) who proposed amending the rule to: 50% of the population had 27% gray hair at age 50 years. Further, these authors compared the age-associated hair graying in 23 regions of the world, over five continents, and as expected, irrespective of ethnic or geographical origin, the hair graying increased with age. At age 45, 57% of subjects were affected by gray hair, which

451 increased with age; at 60 years, 91% of subjects had gray hair. Thus, human hair graying is the
452 time-dependent progressive decline in melanin content in the HS.

453 The color in the HS is due to melanin granules, a mature form of melanosomes
454 furnished by melanocytes of the hair follicle pigmentary unit. The vast majority (95%) of the
455 global population evolved with dark brown or black scalp hair, while 5% have emerged with
456 diverse hair colors such as gray-blonde, yellow-blonde, red, auburn, or shades in between. As
457 reviewed by Itou et al. (Itou et al., 2019), based on the quantitive data of black-dark brown
458 eumelanin and the reddish-brown pheomelanin in different human hairs phenotypes (visual,
459 i.e., black, dark brown, brown, light brown, blond, and red color), a positive correlation exists
460 between chemical phenotype and visual phenotypes. The quantitative measurement of melanin
461 and 5,6-dihydroxy indole (DHI) content in Japanese female hair showed a significant positive
462 correlation with age (Itou et al., 2019). Hair pigmentation depends not only on eumelanin,
463 pheomelanin, and DHI contents but is also significantly impacted by the extent of melanin in
464 hair, its production, and its reservoir. The total regulation of melanin production and reservoir
465 is governed by numerous factors, including hair cycle-dependent variations, gender, ethnicity,
466 hormones, genetics, and age-related changes (Van Neste and Tobin, 2004). The exact
467 mechanism of hair graying remains unclear but is attributed to a time- dependent progressive
468 functional decline in pigmentary machinery, malfunction or loss of the pigmentary unit,
469 melanocyte migration defects, anagen defects, loss of melanocyte cells, or their failure to self-
470 maintain and proliferate (Matsumura et al., 2016; Nishimura et al., 2005). Other studies have
471 linked hair graying with neuroendocrine alterations (Paus, 2011) and oxidative damage (Arck
472 et al., 2006; Paus, 2011).

473 **2.3.1 Omics depicted HS graying biomarkers**

474 Genome-Wide association studies, whole exome sequencing and gene expression
475 studies have identified altered genes in hair graying. It is believed that hair graying occurs with

476 age and its progression is determined by factors like gender, biogeographic ancestry, genetic
477 predispositions and environment/lifestyle factors (Seiberg, 2013; Shin et al., 2015). The genes
478 implicated in hair graying phenotype include loci earlier linked with smoking status (KIF1A,
479 RUNX1, IRF4, BRINP1, TEX41, GRID1), BMI/obesity (GRID1, TEX41, SEMA4D,
480 TBC1D22A, RUNX1), bone mineral density (RUNX1, TBC1D22A, TEX41), cardiovascular
481 diseases (FGF5, MROH2A, DPEP1, GRID1) and immunology (RUNX1, TBC1D22A, IRF4,
482 TMEM132C, GRID1) (Journal of Proteome ResearchPośpiech et al., 2020). The majority of
483 genes identified by genome or exome sequencing are from the hair follicle, hence discussed in
484 detail in the hair follicle section below.

485 To study hair pigmentation patterns in dark, gray, and graying transition (initially dark
486 and then undergoing a sharp graying transition from dark to gray), Rosenberg et al. (Rosenberg
487 et al., 2021) plucked, imaged, digitized, and analyzed them. The electron microscopic analysis
488 of dark hair revealed the distribution of melanin granules throughout the hair cortex. The gray
489 hair from the same individual showed an absence of melanin while, when present, being
490 smaller, less dense, and vacuolated. The transition from dark to gray showed rapid
491 depigmentation events within a single anagen hair cycle (Paus and Cotsarelis, 1999), making
492 it complicated to understand the causes of the complex mechanism of depigmentation.

493 Rosenberg et al. (Rosenberg et al., 2021) adopted a digitization approach to map hair
494 pigmentation patterns in single hairs undergoing graying and also examined proteomic features
495 of both black and gray hair. In this study, these authors collected dark and gray hair from two
496 closely age- and diet-matched individuals. To independently validate these results, the authors
497 further extended black and gray HF analysis from other six individuals (3 males and 3 females,
498 24–39 years) on a separate proteomic platform and in a different laboratory. The proteomic
499 analysis of dark and gray hairs displayed upregulation of proteins associated with ribosome
500 function, protein processing, mitochondrial proteins, and associated cytoskeletal proteins in

gray hair (Rosenberg et al., 2021). Of 34.3% of the upregulated proteins in gray hair, 26.8% were known mitochondrial proteins, and their upregulation can be interpreted as high energy requirements. Further detailed analysis of these proteins suggested the upregulation of specific mitochondrial proteins, not necessarily involved in global energy metabolism but in specific metabolic activities such as amino acid and lipid biosynthesis. Comparison of hair proteome of pigmented and non-pigmented hairs revealed up-regulation of housekeeping gene products, including elongation factor 2 (EEF2), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and enzymes involved in lipid catabolism in non-pigmented hair (Franklin et al., 2020).

The absence of melanin in gray hair, as observed microscopically by Rosenberg et al. (Rosenberg et al., 2021), can be correlated with protein expression. Melanosomes are a special member of the lysosomal lineage and are largely absent in gray hair (Slominski et al., 2005). Rosenberg et al. (Rosenberg et al., 2021) noted a downregulation of lysosomal proteins such as lysosomal phospholipase D (PLD3), cathepsin D (CTSD), beta-hexosaminidase subunit beta (HEXB), and lysosome-associated membrane glycoprotein 1 (LAMP1) in gray hairs and these observations were consistent with other studies by Franklin et al. (Franklin et al., 2020). In addition, these authors noted depletion of KAPs (KRTAP9-6, 4-3, 19-5, 3-1, and 26-1) and downregulation of inter-alpha-trypsin inhibitor heavy chain H4 (ITIH4), beta-2-glycoprotein 1 (APOH), and proteins associated with mitochondrial calcium transmembrane transport in gray hair. The downregulation of these proteins in gray hair suggests a reduction in melanin production.

Using age-matched dark and gray hair samples from European subjects, Franklin et al. (Franklin et al., 2020) found enrichment of protein cathepsin B (CTSB), Sec23B (SEC23B), a vesicular transport protein in dark hair, while elongation factor 2 (EEF2) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were highly abundant in gray hair. While Plott et al. (Plott et al., 2020) noted a higher abundance of tyrosinase-related protein 1 (TYRP1) and

glycoprotein nonmetastatic melanoma protein B (GPNMB) in African Americans compared to a European-American hair sample. These proteins play a key role in melanin biosynthesis (Zhang et al., 2012) and thus reflect higher melanin content in samples from the African American cohort. In summary, hair graying is an apparent sign of aging, resulting from diminished production and deposition of pigments within the HSs. Further, accumulated literature indicates that the proteins involved in general metabolic pathways relevant to aging biology, like carbon metabolism, amino acid biosynthesis, and glycolysis/gluconeogenesis, were enriched in gray hair.

As illustrated in Figure 1, metabolomics is the latest state-of-the-art final "omics" level adopted to discover biomarkers. The plethora of information that can be produced through the "omics" approach provides an opportunity to discover biomarkers or understand hair aging. Metabolomics holds a high potential to elucidate the mechanism of the aging process (Adav and Wang, 2021). Hair metabolomics of melanin-rich black hair and melanin-poor light blond hair depicted hair color dependency on the concentration of N-acetylneuraminic acid and octanoyl carnitine, while no hair color dependency was noted for the relatively neutral amino acid phenylalanine (Eisenbeiss, Binz et al. 2020). The endogenous hair metabolites, like choline, cysteic acid, N-Acetylneuraminic acid, hexanoyl carnitine, octanoyl carnitine, decanoyl carnitine, and theophylline, were highly abundant in black hair than melanin-poor light blond hair (Eisenbeiss et al., 2020b). The nutrients and endogenous metabolites are constantly incorporated into the growing HS, which gets conserved within the stable keratinized hair structure (Eisenbeiss et al., 2020b). The accumulated endo-and exogenous compounds might allow the search for robust biomarkers eliminating dynamic or short-term fluctuations in the metabolic profile. The continuous growth of the HS is advantageous for retrospective and chronological tracking of drugs and metabolite dynamics (Pragst and Balikova, 2006b). Further segmentwise analysis revealed continually decreased signals from

proximal to distal for most metabolites, including choline and decanoyl carnitine (Eisenbeiss et al., 2020b). Noppe et al. (Noppe et al., 2015) noted a gradual decrease in steroid concentrations from proximal towards the distal end of the HS after the segmentation of hair in 3 cm segments. The segment analysis of the hair sample is useful in determining the age of the specific metabolite or duration of exposure to a particular drug. Further efforts are required to correlate metabolic changes to diet and lifestyle habits, including smoking behavior, alcohol habits, and diet. In summary, even though hair metabolomics is in its early stage, with a reasonable study design and appropriate data interpretation, hair metabolomics has the potential to elucidate hair aging, dietary or lifestyle habits, and drug exposure.

3. HAIR FOLLICLE - OMICS ADVANCES

Hair follicles (HFs) can be thought of as mini-organs in the skin, generally formed during fetal and perinatal skin development, whose further development is governed by neuroectodermal-mesodermal interactions (Schmidt-Ullrich and Paus, 2005). HFs reside in the dermal layer of the skin and consist of the dermal papilla (DP) at the base, surrounded by matrix cells that proliferate and differentiate to form the HS. HF, an epithelial appendage, has two unique fibroblast populations: the dermal papilla (DP) and the dermal sheath (DS). The DP is critical in hair regeneration and is required for induction, growth, and maintenance (Yang and Cotsarelis, 2010). The DP is separated from surrounding matrix epithelial cells by an epidermal basement membrane and connected at its base to the DS cup cells (Fig. 2). DP encapsulates each follicle up to the sebaceous gland (Yang and Cotsarelis, 2010), and these cells migrate from the HF to repopulate the surrounding dermis after wounding (Jahoda and Reynolds, 2001). The supra-bulbar region of the follicle comprises the outer and inner root sheath consisting of Henle's layer, Huxley's layer, and an internal cuticle that continues with the

outermost layer of the hair fiber (Fig. 2). The outer root sheath extends from the epidermis to the hair bulb, which houses several types of stem cells. The outer root sheath of the hair follicle contains melanocytes. The detailed anatomy and physiology are described elsewhere (Erdoğan, 2017; Schneider et al., 2009).

Being a mini-organ, HF offers a unique perspective for aging research (Sikkink et al., 2020). HF presents an elegant developmental system since its cells get replenished regularly under controlled proliferation, lineage specification, and terminal differentiation. Consequently, HF biology is attracting tremendous interest with extremely diverse study approaches aiming to understand the complex mechanisms of aging, HS aging and graying, biomarkers discovery, HF regeneration and investigating therapeutic strategies to delay aging (Takahashi et al., 2020; Wu et al., 2022; Zheng et al., 2022). .

Human aging is very complex and numerous theories of aging have been proposed (Davidovic et al., 2010; Jin, 2010) including the "theory of longevity" which is based on the "on" and "off" states of certain genes, López-Otín (López-Otín et al., 2013) proposed nine hallmarks of aging - genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient-sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion and altered intercellular communication, that represent common denominators of aging in different organisms, with an emphasis on mammalian aging. Recently, the "Information Theory of Aging" has been proposed which postulates that "aging in eukaryotes is due to the loss of genetic and epigenetic information over time" (Yang et al., 2023). According to David Sinclair and his colleagues (Yang et al., 2023), epigenetic information is lost over time due to the relocalization of chromatin-modifying proteins to DNA breaks, causing cells to lose their identity. There are numerous aging theories which are discussed in-depth in articles including those cited herein, and will not be dealt with in this Review.

The genetic predispositions in hair graying are debatable since Adhikari et al (Adhikari et al., 2016) showed 27% heritability in predicting hair graying while others found this to be 90% (Shin et al., 2015). Such a significant difference in genetic prediction of hair graying susceptibility may be due to differences in the techniques adopted by different authors. The genome-wide association studies in Latin Americans revealed the association of gene IRF4 with hair graying (Adhikari et al., 2016). This IRF4 encodes interferon regulatory factor and regulates MITF transcription factor which plays a key role in the expression of the TYR gene encoding tyrosinase, a key enzyme in melanin synthesis (Praetorius et al., 2013). Exome-wide association analysis identified two novel DNA variants rs59733750 in KIF1A and rs1127228 in NSMCE1, to be associated with hair graying (Fujioka et al., 2002). Further, exome-wide identified locus, NSMCE1 (16p12.1), improved the accuracy of hair graying prediction (Journal of Proteome ResearchPośpiech et al., 2020). Other studies also found significant differences in transcription-coupled nucleotide excision repair (NER) signaling pathways, which coordinate genome stability, DNA repair, gene transcription, as well as damage tolerance, in gray and black hair (Fousteri and Mullenders, 2008).

Immunohistochemical evaluation of interfollicular scalp on a population of 650 volunteers (300 women and 350 men) for over 7 years demonstrated shrinking in the microvasculature, reduction in versican+ cells, and the number of CD117+ mast cells with advancing age (Piérard-Franchimont et al., 2013). The extracellular matrix (ECM) provides the dermal structure and integrity and is maintained by interfollicular dermal fibroblasts (DFs) (Kalluri and Zeisberg, 2006). However, collagen fibrils get disorganized and fragmented with advancing age. In the aged skin, matrix metalloproteinase 1 (MMP1), which possesses the capacity to degrade native fibrillar collagen, was found to be elevated (Quan et al., 2009). Age-related changes in human scalp ECM, including reduced versican (VCAN) expression with age, have been documented (Piérard-Franchimont et al., 2013; Williams et al., 2021). A major

ECM proteoglycan hyaluronic acid (HA) essential for maintaining skin hydration is synthesized by HA synthases (HAS) (Baumann, 2007), and HAS2 was downregulated with age in cultured DF, which can be interpreted as reduced HA synthesis.

Jo et al. (Jo et al., 2016) compared histological features of the scalp skin from the occipital promontory area sampled from young (average age, 33.3 years) and old (average age, 83.8 years) males to investigate aging-associated changes in HFs and found an age-associated decrease in versican levels in the follicular DPs. LC-MS analysis of the secretome of DFs from young (19–29 years) and older (53–57 years) women revealed significant differences in the abundances of 26 proteins (Williams et al., 2021), including upregulation of cartilage oligomeric protein (COMP), α-smooth muscle actin (αSMA), and the glycosylated SPARC (secreted protein, acidic and rich in cysteine). No age-associated regulation was observed in the abundance of αSMA and SPARC. Thus, an aging scalp shows remarkable structural and biological alterations in the hair follicle environment including epidermal thickness and rete ridges and changes in sebum secretion, expression of proteins affecting cell migration, dermal sheath proteins. In summary, age-related changes in the surrounding scalp skin, including changes in sebum secretion and other biochemical changes, may initiate a reduction in follicle size, which may further lead to fiber thinning. As stated earlier, HF is a mini organ in the skin consisting of the dermal papilla, matrix cells, melanocytes, dermal sheath, etc. Thus the impact of aging on these constituents needs to be considered.

3.1 Dermal papillae

Dermal papillae (DP) are a specialized mesenchymal component of hair positioned at the base of the HF and are enveloped by the dermal sheath in the hair bulb of an HF (Fig. 2). Dermal papillae cells (DPCs) are growth factor reservoirs specialized in hair morphogenesis and regeneration They co-ordinate hair follicle growth and cycling, and act as control centres

for the hair cycle. HFs are composed of more than 20 cell types, but DPCs are central in growth factor production, supply and regulation (Kim et al., 2021a). As a result, DPCs are considered therapeutic targets in preventing hair thinning and loss. Human follicle dermal papilla (HFDP) was used to evaluate the impact of anti-aging porphyra-334 using transcriptomics and upregulation of metallothionein- (MT-) associated genes, particularly MT1E, MT1L, MT1X, and MT2A genes were noted (Kim et al., 2021b). Further, these authors noted several genes associated with the hair follicle cycle, the hair follicle structure, the epidermal structure, and stem cells being upregulated by porphyra-334 treatment. Aging alterations in the DP structure have been investigated in several areas of the body and it was noted that a decrease in number, increase in cross-sectional area, and decrease in the height of the DP have all been observed with aging (Imbert et al., 2012; Lagarrigue et al., 2012). The accumulated literature suggests that the DP structure increased in numbers from age 0 to the thirties and decreased after 40 years of age. Even though DPCs are crucial, the impact of age on DPCs themselves has been rarely explored.

About 50 years ago; it was shown that DPs could be extracted from the hair follicle and transplanted into the recipient's skin, which induces follicle development and hair growth (Cohen, 1961). These findings suggest that DP can reprogram the non-hair-bearing epidermis to a follicular fate, reiterating the events of embryonic hair morphogenesis. However, transplanting DPs was hindered since culturing them in 2D culture imposed unnatural constraints with altered cellular behavior and loss of their ability of hair-follicle induction (Birgersdotter et al., 2005). The tendency to aggregate remains a key characteristic of dermal papilla cells (DPCs), but this aggregation property and HF induction were lost after a few passages of culture (Zhang et al., 2014). The loss of inductive potential was considered to be associated with the loss of ECM and ECM proteins (Balañá et al., 2015). These limitations were later overcome using a spheroid aggregate culture that preserved their structure

morphologically and biochemically (Higgins et al., 2010). By creating 3D spheroids, researchers (Higgins et al., 2013) have restored gene-expression signatures within the cells and associated hair-inducing properties of DP.

To delineate the possible role of ECM and ECM proteins in inducing HF differentiation, secreted proteins between early- and late-passage DPCs cultures were quantified by iTRAQ-based quantitative proteomics (Zhang et al., 2016). Using iTRAQ, these authors quantified 1360 ECM proteins, of which 213 proteins were differentially expressed in ECM from early-passage vs. late-passage DPCs. The detailed analysis of these proteins revealed ECM from aggregated DPCs and early-passage DPCs, exclusively inducing HF regeneration. No HF induction was noted in non-aggregated DPCs or ECM from late-passage DPCs. KEGG pathway and enrichment analysis of these proteins revealed the TGF-β signaling pathway as essential for regeneration, as it activates the Smad2/3 pathway in HF (Rishikaysh et al., 2014). The molecular mechanism involves activation of the canonical Wnt signaling through Stromelysin-1 (MMP3), where stromal cell-derived factor 1 (SDF1) and biglycan play a major role (Zhang et al., 2016).

The secretory factors of DPCs also control and regulate the hair growth cycle (Rosenquist and Martin, 1996). The volume of the DPCs has been correlated with follicle size (Elliott et al., 1999) and attributed to the extracellular matrix (ECM), and hence thicker terminal hair follicles have a higher abundance of ECM. During anagen, HF produces hair fibers and then enters into the catagen phase, in which shrinking of HFs and remodeling of the ECM lead to the release of DPCs from hair bulbs. The DPCs become invisible in the telogen follicle, and a new ECM needs to be synthesized at the onset of the following anagen phase. The variation and abundances of the ECM protein, including fibronectin, chondroitin sulfate proteoglycan, and growth factors, suggest that these molecules may play a critical role in epithelial-mesenchymal interactions within the hair follicle (McElwee et al., 2004). The accumulated

699 literature suggests that it is the DPCs that secrete diverse growth factors and induces
700 proliferation and differentiation of follicular epithelium, control the development of hair
701 follicles, regulate hair formation, hair growth, and cycling through paracrine mechanism
702 (Pflieger et al., 2006; Schmidt-Ullrich and Paus, 2005; Zhang et al., 2014). However, the
703 impact of the aging dermal environment on scalp HF, DPCs, their secretions, and the
704 abundance of the secreted components of ECM remains unclear. Hence, it is important to
705 establish both the secretome profile of DPCs and age-dependent differences in it to understand
706 hair follicle development, get novel insights into the hair growth process, functional proteins
707 in hair biology, and the clinical application of relevant protein biomarkers.

708 Won et al. (Won et al., 2012) adopted shotgun proteomics to profile the secretome of
709 human dermal papilla cells (DPCs) and matched dermal fibroblasts (DFs), and identified 1188
710 proteins in DPCs and 1271 in DFs. Of these proteins, 28 proteins were DPCs-specific ECM
711 proteins, including transporters (ECM1, A2M), enzymes (LOX, PON2), and peptidases (C3,
712 C1R). Based on these identified proteins' biochemical and network analysis, Won et al. (Won
713 et al., 2012) proposed ITGB1, IGFBP3, and THBS1 as hair-growth-modulating protein
714 biomarkers. Meanwhile, by adopting the LC-MS approach, Pflieger et al. (Pflieger et al., 2006)
715 discovered a higher level of transforming growth factors such as beta-induced protein IG-H3
716 (BIGH3), fibronectin, and thrombospondin 1 (TSP1) in the secretome of DPCs. The critical
717 role of TSP1 in the induction of anagen follicles has been well established (Yano et al., 2003).
718 ITGB1 remains a potential hair follicle-modulating factor and plays a key role in cell adhesion,
719 migration, and survival (Carlson et al., 2008). Similarly, Stenn and Paus (Stenn and Paus, 2001)
720 concluded that the DPCs secrete distinct growth factors and stimulate the proliferation and
721 differentiation of the follicular epithelium.

722 **3.2 Melanocytes**

723 The hair follicle is the most aging-resistant organ, with the prominent exception of the
724 pigmentary unit. The hair follicle pigmentary unit is perhaps one of the most age-sensitive;
725 hence, visible changes in the HS pigment intensity appear long before even slight alterations
726 are seen in the epidermis. Melanin production occurs via a biochemical pathway called
727 melanogenesis in unique lysosome-related organelles termed melanosomes, whose biogenesis,
728 maturation, and trafficking occur within the cytoplasm of cells called melanocytes.
729 Melanocytes are located at different sites, including the basal layer of the epidermis, among
730 the basal sebocytes of the sebaceous gland, and in the midportion of the hair follicle outer root
731 sheath. However, the sub-population located in the hair bulb above and around the mid-upper
732 follicular papilla only contributes to the pigmentation of the HS (Van Neste and Tobin, 2004).
733 The melanocytes residing in hair follicles remain tightly regulated during hair follicle cycling,
734 while epidermal melanogenesis appears independent of hair follicle cycling (Sarin and Artandi,
735 2007).

736 A very fine unpigmented lanugo hair appears during fetal life, which further changes
737 to vellus hair during childhood and then a longer, thicker dark, pigmented hair at puberty, and
738 thus the hair follicle pigmentary units are susceptible to age-related changes (Costin and
739 Hearing, 2007). Hormones like androgens and estrogens also influence age-associated hair
740 color change. Thus melanocytes are sensitive to (neuro)endocrine factors (Tobin and Kauser,
741 2005). The spectacular range of hair colors is a function of variable relative amounts of
742 brown/black eumelanin and yellow/red pheomelanin. The biology of the hair follicle (Paus and
743 Cotsarelis, 1999; Schneider et al., 2009) and the development of the pigmentary unit have been
744 extensively reviewed (Botchkareva et al., 2003; Slominski et al., 2005; Tobin, 2009).

745 In a single hair growth cycle, a relatively small number of melanocytes produces
746 enough melanin to pigment up to 1.5 m of the HS only during youth (15–24 years) (Tobin and

747 Paus, 2001). Melanosomes synthesize both melanins (eumelanin and pheomelanin) by a series
748 of reactions (Fig. 3) catalyzed by specific melanogenic enzymes, such as tyrosinase, tyrosinase-
749 related protein 1 (tyrp1) and tyrp2 (Ito and Wakamatsu, 2011). In the biosynthesis of melanin,
750 tyrosine serves as the initial substrate, while tyrosinase remains the rate-limiting enzyme. Ito
751 and Wakamatsu (2011) explain that dopaquinone undergoes an intramolecular reaction of the
752 amino group to give dopachrome via cyclodopa when the amino acid cysteine is not available
753 in melanosomes. Dopachrome spontaneously gets converted into 5,6-dihydroxy indole (DHI),
754 but such conversion can be augmented by dopachrome tautomerase (DCT), also called
755 tyrosinase-related protein-2 (TYRP2), which catalyzes the tautomerization of dopachrome to
756 5,6-dihydroxyindole-2-carboxylic acid (DHICA). The oxidative polymerization of DHI and
757 DHICA gives eumelanin. The biochemical pathway leading to the formation of pheomelanin
758 involves the synthesis of cysteinyl dopa, a condensation product of dopaquinone and the amino
759 acid cysteine. During HS melanin production, the precise interactions in the hair follicle
760 pigmentary unit involving follicular melanocytes, keratinocytes, and dermal papilla fibroblasts
761 facilitate melanogenesis regulation (Slominski et al., 2005). The regulation of melanogenesis
762 through different transporters and the roles of different proteins involved in melanogenesis
763 have been discussed and reviewed (D'Mello et al., 2016; Ito and Wakamatsu, 2011; Rishikaysh
764 et al., 2014).

765 An age-associated loss of pigment in the hair can be much more dramatic, while it is
766 rather gradual in the epidermis, which indicates heterogeneity in melanocytes and reflects
767 differences in their intrinsic ‘melanogenetic clocks’. It has been demonstrated that epidermal
768 and HF melanocytes express clock genes/proteins in culture (Sandu et al., 2012). The clock
769 genes directly regulate the anagen-to-catagen transition in organ-cultured human scalp HF
770 without central clock inputs (Al-Nuaimi et al., 2014). Thus, the molecular clock is an integral
771 component of the “hair cycle clock”. However, melanogenesis is coupled with anagen. Hence

772 the molecular clock could be a candidate for timing the on/off switching of HF pigmentation.
773 Hardman et al. (Hardman et al., 2015) silenced the clock genes PER1 and/or BMAL1 and found
774 increased melanin content and expression of both TYRP1 and TYRP2 in human HF. Thus,
775 silencing clock activity promotes human HF pigmentation in a hair cycle-independent manner.
776 In other words, these clock genes can be an excellent therapeutic target to exploit the topical
777 application of small molecule modulators of clock proteins to manage HF pigmentation.

778 Melanocyte aging may first dominate the hair aging process at the gene expression
779 level. According to Baxter et al (Baxter et al., 2019) and further recent updates by the
780 International Federation of Pigment Cell (http://www.espcr.org/micemut/), 68 genes were
781 linked to hypopigmentation or depigmentation. Genes like TYR (rate-limiting enzyme for
782 pigment production), TYRP1(melanin-processing), TYRP2, MITF, Pax3, Sox10
783 (melanogenesis), Plexin C1(axon guidance), Melan-A (melanosome structure and Pmel17
784 regulator) and Pmel17 (structural protein central for early melanosome maturation) play key
785 roles in the development of melanocytes and melanogenesis with age (Peters et al., 2013).
786 These authors obtained intriguing data from intra-individual sample comparisons and proposed
787 the graying HF as a valid aging model and a promising target for testing therapeutic
788 interventions. Transcriptional profiling of HF revealed altered expression of melanocyte
789 biology–associated markers and regulated pathways that are indicative of a role in the HF aging
790 process. This study emphasized concentration of glutamine in maintaining hair growth and
791 pigmentation, suggesting suboptimal concentrations promote an aging-like process in cultured
792 HF. While evaluating melanocyte subpopulation turnover, Commo and Bernard (Commo and
793 Bernard, 2000) noted a decrease in pMel17 with aging which proved that hair graying with age
794 happens in tandem with a specific and gradual decline in the total number of melanocytes in
795 individual hair follicles. Subsequently, a study by Choi et al (Choi et al., 2008) found a
796 significant decrease in the expression of the MITF-M, Sox10, Pax3, TYR, TYRP1, and TYRP2

genes in the hair bulbs of white hair compared to black hair. Peters et al (Peters et al., 2013) also observed a stepwise sequential decrease in TYRP-1, TYR, Pmel17, Melan-A (Pmel17 regulator), KIT (tyrosinekinase receptor for stem cell factor), and MET (tyrosinekinase receptor for hepatocyte growth factor) in gray HFs, which can be interpreted as depletion of melanocytes during aging. The genetics of hair pigmentation (Pavan and Sturm, 2019) and genetics of graying hair with age (Wang et al., 2023) have been well-reviewed, hence, not discussed in detail in this article.

The study of melanocyte aging in hair has inspired much more interest in melanocyte stem cells since melanocytes are maintained by a small number of stem cells residing in the bulge region of the hair follicle (Sarin and Artandi, 2007). The loss of melanocytes and melanocyte stem cells has been linked with the loss of hair pigmentation in old individuals (Steingrímsson et al., 2005). The mutation of TYRP1 leads to loss of function or ginger-red hair, particularly rufous albinism (Morice-Picard et al., 2014). This mutation caused a decline in tyrosinase activity, catalase activity (Kauser et al., 2011), and loss of TYRP2 expression in HF melanocytes (Commo et al., 2004). Melanogenesis is responsive to hair cycle-dependent changes and racial and gender differences, hormones, genetic defects, and age-associated changes (Tobin, 2005). The growth factors, cytokines, hormones, neuropeptides and neurotransmitters, eicosanoids, cyclic nucleotides, nutrients, microelements, and cations/anions also impact melanogenesis (Slominski et al., 2004). In short, hair graying is mostly characterized by the loss of pigment, but the total events, their control, and regulation with age are still unknown.

3.3 Sebaceous glands

Sebaceous gland activity is critical for proper skin function and is typically associated with HF. During differentiation, sebocytes synthesize and accumulate specific lipids, which constitute the major content of sebum. They undergo apoptosis and release their entire content

into the sebaceous duct that opens into the canal of HF. With aging, sebocyte proliferation, intracellular lipid synthesis, sebum transit time in the HF, storage in the infundibulum reservoir, etc., become altered (Piérard et al., 2000). The number of sebaceous glands remains approximately the same throughout life. However, their size increases with age, reaching a maximum at ~30 years old, remaining constant during middle age, and then decreasing (Zouboulis et al., 2003). With age, a decrease in the sebum secretion rate and a decline in the activity of the sebaceous gland have been documented (Rittié and Fisher, 2015).

Yamamoto et al. (Yamamoto et al., 1987) adopted gas chromatographic techniques and investigated sebum samples of 55 healthy individuals to investigate the impact of aging on the sebaceous gland. The sebaceous gland activity was monitored using a ratio of wax esters/(cholesterol + cholesterol esters [i.e., WE/ (C + CE)], which exhibited peak activity at the age of 20 years and decreased after that in the individuals in their 30s and 40s. These authors established the relationship between C16:1 straight and C16:1 iso-branched chain fatty acids, where the former increased in proportion from infancy, peaked in the 20s, and decreased until the 50s. Interestingly, mass spectrometric analysis of SZ95 sebocytes identified 874 proteins in lipid droplets, of which 54 proteins were significantly enriched (Dahlhoff et al., 2015). The enriched proteins include proteins involved in membrane trafficking such as Rab family members (RAB5A, RAB5C, RAB7A), protein degradation pathway (AUP1, UBXN4), lipid metabolism (ACSL3, CYB5R3, LPCAT1, LPCAT2, LPCAT4, etc.), and perilipins (PLIN2, PLIN3). The high abundance of PLIN2 and PLIN3 in SZ95 sebocytes was documented (Camera et al., 2014; Dahlhoff et al., 2013), and it was shown that the key pathways of neutral lipid formation are under the influence of the PLIN3 (Camera et al., 2014).

The expression of the murine Foxc1 gene in the hair follicle stem cells and sebaceous glands has been documented, and loss of expression of Foxc1 or the loss of the function of this gene results in hyperactivation of the hair cycle phases, premature hair aging, and decay in the

hair follicle (Lay et al., 2016). The deletion of this gene results in the loss of hair (Hariri et al., 2018). The spontaneous mutation on chromosome 11 close to the type I keratin locus resulted in abnormal sebaceous gland differentiation, a defective hair cycle, and shorter than normal HS (Rebecca et al., 2002). Age and sun exposure harbors the mutation burden (Martincorena et al., 2015), which may accelerate the aging-associated alterations over time.

3.4 Eccrine, apocrine, and apoeccrine glands

Eccrine, apocrine, and apoeccrine glands are considered as sweat gands. Of these, eccrine sweat glands (ESGs) are numerous (about 2–4 million), distributed across nearly all regions of the skin and play key roles in thermoregulation via evaporative heat loss (Poblet et al., 2018). Based on the excretion of sweat, ESGs produce the highest volume while apocrine and apoeccrine glands play a lesser role in overall sweat production as they are limited to specific regions of the body (Baker, 2019). Although Eccrine glands are numerous, their density is not uniform across the body surface. The highest gland densities are found on the palm and sole where they respond to emotional as well as thermal stimuli. Apocrine sweat glands are located primarily in the axilla, breasts, face, scalp, and perineum. The apocrine glands are larger and open into hair follicles while eccrine glands are smaller and open onto the skin surface (Bovell, 2018). In addition, although present from birth, the secretory function of apocrine glands does not begin until puberty and the secretome is mostly viscous and lipid-rich. It also contains proteins, sugars, ammonia and pheromones. On the contrary, eccrine sweat is mostly water and NaCl, but also contains a mixture of metabolites originating from the interstitial fluid and the eccrine gland itself. Apoeccrine glands develop from eccrine sweat glands between the ages of ~8 to 14 years and are present only in the axillary region. Apoeccrine glands are more similar to eccrine glands, where their distal duct connects to and empties sweat directly onto skin surfaces and they produce salt-containing sweat. Upon increasing internal temperature of the body, sweat glands release water onto the skin surface

872 that quickly evaporates, thus cooling down the skin and blood beneath. These glands play a
873 key role as a thermoregulator. At the same time, it is important to note that sweat is another
874 way of expelling metabolic wastes besides urine. The detailed anatomy, physiology and
875 functions of these sweat glands and sweat composition are reviewed elsewhere (Baker, 2019).
876 Na et al (Na et al., 2019) isolated eccrine sweat glands from human skin by
877 microdissection, performed RNA-seq and proteome analysis, and identified ∼138,000
878 transcripts and ∼6,100 proteins. Proteins including chloride intracellular channel protein,
879 sodium potassium-transporting ATPase subunit, sodium bicarbonate transporter, voltage-gated
880 potassium channel, calcium-activated potassium channel, voltage-dependent calcium channels,
881 V-H+-ATPase, AQP5 and carbonic anhydrases were detected in the eccrine sweat glands.
882 Other researchers (Bovell et al., 2011; Clunes et al., 2004) also identified proteins belonging
883 to Na+K+ATPase, K+ channels, Ca+2 channels, V-H+-ATPase, and AQP5 in eccrine sweat
884 glands. However, further studies are required to evaluate variabilities between individuals of
885 different genetic backgrounds, sex, and age. Profiling the composition of eccrine sweat glands
886 may provide a better understanding of conditions such as cystic fibrosis, hyperhidrosis,
887 anhidrosis, and atopic dermatitis, as well as the regrowth of sweat glands in burn victims
888 (Doolittle et al., 2016; Ma et al., 2016). When the eccrine sweat gland transcriptome was
889 compared to that of other human tissues/cells, the highest similarity was found in comparison
890 to the cortex of the kidneys, which emphasizes the eccrine sweat gland's secretory function
891 that is similar to the kidney. Based on the level of urea excreted by sweat glands, it is believed
892 that there should be urea transporters in sweat glands. As expected, Aquaporin-5(AQP5) and
893 renal urea transporters UT-A and TU-B were identified in sweat glands (Xie et al., 2017).
894 The skin is mostly a hostile environment for nucleic acids because of the presence of
895 nucleases. However extracellular vesicles (EVs) extracted from human sweat indicated the
896 presence of nucleic acids. Of the human sweat-extracted DNA, the most represented mRNA

were mitochondrial origin, suggesting the release of mitochondria during oxidative stress (Hayakawa et al., 2016) and subsequent transport in EVs (Hough et al., 2018). Bart et al (Bart et al., 2021) collected sweat from 13 volunteers of both gender aged from 26 to 56 years after biking exercise. They extracted EVs, characterize DNA and RNA using next-generation sequencing and found a wealth of nucleic acids, including DNA and RNA of human and microbial origin. These authors found 5% reads resembling genomes belonging to bacteria, archaea, and viruses. Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes, which are usually found on human arms, hands, and axilla were identified. Such microbiomes have been used in forensic sciences for the identification of individuals, geolocation inference, post-mortem interval (PMI) estimation, and others (Swayambhu et al., 2023; Zhang et al., 2022a).The predominance of RNA, tRNA, miRNA, LincRNA, rRNA and miscRNA was observed in many other EV-extracted RNA studies (Bart et al., 2021; Wei et al., 2017) and linked to cardiac arrhythmia and sudden death of young people after exercise or exertion related death (Anderson et al., 2016).

Human scalp dermal gray adipose tissue (dWAT) surrounds the HF and eccrine sweat glands and extends up to the bulge region (Poblet et al., 2018). The impact of perifollicular adipocytes on the physiology of human anagen scalp HFs has been investigated and it was noted that the number and size of perifollicular adipocytes declined during anagen–catagen transition (Nicu et al., 2021). These authors further found increased lipid oxidation in adipocytes surrounding the bulge and/or sub-bulge region. Using a combination of immunohistomorphometry, X-ray microcomputed tomography, transmission electron microscopy and Laser-capture microdissection–based microarray analysis, Nicu et al (Nicu et al., 2021) demonstrated the mechanism of communication and concluded that perifollicular adipose tissue modulates human HF growth and pigmentation through HGF/c-Met signaling. According to Zwick et al (Zwick et al., 2018), dWAT provides essential factors modulating

periodic HF growth, responds to skin bacterial infection, and promotes wound healing. The relationship between dWAT and HF has also been established in mouse models (Festa et al., 2011). During aging of HF, dWAT repressed HF cycling behavior in aging mice but by transplanting aging skin into young mice, the defective HF recovered under the modulation of young dWAT(Chen et al., 2014). Alterations of dWAT with aging, its regulatory role, and the effects of dWAT on aging HFs have been well established (Chen et al., 2021). Since sweat glands, sebaceous glands and HF are embedded in the dermal layer , their three-dimensional (3D) spatial-functional relationship remains complex. Age-dependent alterations in the dermal layer result in loss of skin elasticity, impaired wound healing potential and cause wrinkling and sagging (Shin et al., 2019). The 3D structure of the sweat gland coupled with X-ray microCT methodologies revealed subcutaneous adipose tissue infiltrating into the dermal layer and decreasing type 1 collagen and elastin expression with aging, which are a major contributing factor to loss of skin stiffness and elasticity (Ezure et al., 2022). Previously, (Ezure and Amano, 2012) showed a decrease in the elasticity of the dermal layer with age which is consistent with others (Mizukoshi et al., 2021; Sakata et al., 2018).

4. IMPACT OF INTRINSIC AND EXTRINSIC FACTORS ON HAIR AGING

The scalp and hair are subjected to intrinsic and extrinsic aging. Hair and scalp aging are impacted by intrinsic factors, including those associated with individual genetic and epigenetic mechanisms, and extrinsic factors, such as ultraviolet radiation (UVR), pollution, smoking, nutrition, lifestyle, and cosmetic hair treatments. Thus, aging remains a consequence of two independent biological processes, one programmed loss of functionality and other damage-related changes. The damage-related theories, including wear and tear theory, cross-linking theory, and free-radical theory, emphasize environmental assaults to cells\tissues that

induce cumulative damage at various levels. The free radical theory of aging was initially proposed by Harman in 1956 (Harman, 1956; Jin, 2010) and is most widely accepted to explain biological aging, including the hair follicle (Arck et al., 2006).

The free radical theory hypothesizes that aging is caused by the accumulation of oxidative damage from reactive oxygen species (ROS), partially reduced metabolites of molecular oxygen generated as products of metabolic reactions or by-products of various cellular processes. Both endogenous reactive nitrogen species (RON) and reactive oxygen species (ROS) are generated during normal metabolism and externally through exposure to various environmental oxidative stresses. The endogenous reactive oxygen and nitrogen species (ROSN) production is vital for many cellular signaling processes within the hair follicle, but the ROS and RON levels need to be maintained below a certain level to prevent cellular and macromolecule damage. These species or free radicals are highly reactive and can directly damage lipids, proteins, and DNA, which are fundamental molecules. The negative effects of ROS are neutralized by antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. However, the imbalance between RONS production and antioxidant defenses results in oxidative stress and oxidative damage.

The “free radical theory of graying” proposed by Arck et al (Arck et al., 2006) adopted macroscopic and immunohistomorphometric techniques and found accumulation and deleterious effects of oxidative stress, which damaged melanocytes of graying HFs and resulted in mitochondrial deletion impacting the functions of the respiratory chain. Further, melanocyte apoptosis and increased oxidative stress in the pigmentary unit of graying HFs were noted. Thus, higher oxidative stress in HF melanocytes leads to their selective premature aging and apoptosis. Similar histopathological analysis of HF showed a decrease in hair bulb melanocytes and eventually their complete loss (Fernandez-Flores et al., 2019). Recently, hair aging research has indicated oxidative stress as a major cause of “hair graying” (O'Sullivan et al.,

971 2021). Upon inducing oxidative stress by exposing HF and scalp skin to free radicals, De
972 Tollenaere et al. (De Tollenaere et al., 2021) found the down-regulation of various genes
973 (MITF, TYR, DKK1, EDN1) linked to melanin synthesis and genes encoding for
974 melanogenesis-related functions, such as melanosome formation and transfer (CTNS, HPS5,
975 MLANA, MLPH, MYO5A, SLC24A5). Further, this study observed a significant negative
976 impact of oxidative stress on the expression of genes involved in antioxidant activities (FTL,
977 GLRX, HMOX1, MGST1, PRDX1, and SOD2). COX2 and KRT19 were down-regulated and
978 consistent with the findings by Shi et al. (Shi et al., 2014). The findings from different studies
979 (De Tollenaere et al., 2021; O'Sullivan et al., 2021; Shi et al., 2014; Tang et al., 2012) can be
980 summarized as oxidative stress impacts the functions of the hair follicle, the process of
981 melanogenesis, and hair aging. The critical factors that impact the melanocytes and pigmentary
982 unit are oxidative damage, age-associated changes in cell survival signals, the balance of self-
983 renewal and differentiation, and the limited lifespan of melanocytes (Sarin and Artandi, 2007).
984 The extraordinary melanogenic activity of pigmented bulbar melanocytes is likely to
985 generate enormous amounts of ROS via the oxidation of tyrosine and dopa to melanin
986 (Hegedus, 2000), which may lead to an imbalance between ROS production and antioxidant
987 systems. The endogenous defense mechanisms, such as antioxidant systems within the HF
988 melanocyte, become weak with age, leading to uncontrolled damage to the melanocyte itself
989 from its melanogenesis-related oxidative stress (Trüeb, 2015). The decreased number of viable
990 melanocytes in the aging hair follicle and oxidative stress associated with mitochondrial DNA
991 damage have been documented (Arck et al., 2006). Together, these findings provide unique
992 evidence of oxidative stress-induced loss of melanocytes from the human HF during aging and
993 support the proposed hypothesis of a “free radical theory of graying”. Other factors like genetic,
994 psychoemotional, senescence- associated, (neuro-)endocrine, metabolic, and nutritional
995 factors (O'Sullivan et al., 2021) impact the pigmentary unit.

Wood et al. (Wood et al., 2009) have identified the existence of hydrogen peroxide (H_2O_2), its oxidation products, and the low level of catalase enzyme in the native senile human gray/gray anagen HS. These authors demonstrated H_2O_2-induced oxidative damage in the entire human HF, including the HS, and emphasized oxidative damage as a cause of senile hair graying. Other studies noted H_2O_2 as a byproduct of melanin synthesis and following UV irradiation (Jiménez-Cervantes et al., 2001). In addition to the melanogenic activity of pigmented melanocytes, the source of oxidative stress also includes oxidative metabolism, smoking, UVR, chemical insults, oxidized scalp lipids, pollutants, inflammation due to microbial imbalance, and hair diseases such as androgenetic alopecia and senescent alopecia (Trüeb, 2015). Melanocyte apoptosis and DNA damage due to H_2O_2-mediated stress have been well-established (Arck et al., 2006). Under H_2O_2-mediated stress, the functions of tyrosinase, the key enzyme involved in melanin production, and other proteins, including antiapoptotic Bcl-2 protein, might be impacted since these proteins are possible targets of oxidative damage (Trüeb, 2015). It is important to note that H_2O_2 at low concentration ($\leq$ 0.3 mM) activates tyrosinase activity and promotes the proliferation and migration of melanocytes. However, at higher concentrations (>0.3–10 mM), these activities get downregulated, including the deactivation of tyrosinase activity (Tang et al., 2012). Thus, oxidative stress and antioxidant balance are crucial in melanogenesis.

Extensive literature related to HF aging found oxidative stress to be a major risk factor for the aging of the pigmentary unit and HF. HF is well equipped with anti-aging components like effective antioxidant potential (Fischer et al., 2008), ROS scavenging and DNA repair enzymes, oxidative damage response controls such as NRF2 activity (Slominski et al., 2018), intrafollicular synthesis of neurohormones including thyroid-stimulating hormone and thyroid-releasing hormone that augments mitochondrial function and activity (Paus et al., 2014). Yet, it is still unclear why the HF pigmentary unit is so sensitive to oxidative damage and ages

unusually fast. The possible mechanism could be explained through telomerase activity of hair matrix epithelium during anagen phase. The resistance of HF epithelial stem cells to detrimental effects of aging might originate in telomere-dependent pathways (Aviv and Shay, 2018). However, major work related to HF telomerase, telomere shortening and telomere dynamics etc., were done in mice (Jan et al., 2012; Siegl-Cachedenier et al., 2007; Tejera et al., 2010) and little is known in human HF. Recent literature on telomerase activity and telomere length dynamics in HF, and how they impact HF aging have been already reviewed by Stone et al (Stone et al., 2021). These authors have explained the clinical importance of telomerase biology in the human HFs, delineated potential therapeutic strategies that target telomere length dynamics in human HFs and emphasized telomere dynamics and telomerase activity in the biology of HFs and their stem cells as a model of aging research. The human HFs obtained during hair transplant surgery can be microdissected, organ cultured, and used for *in-vitro* or *in-vivo* research. Such research will enable in-depth insights into possible pathways of regulation of HF proliferation and aging, and the role of telomerase in these processes.

The mammalian HF contains several stem cell populations and can be a key model for the dissection of collaboration and interaction among distinct cell types. The HF bulge maintains epithelial stem cells (EpSCs) and melanocyte stem cells (McSCs) which interact with each other and undergo activation and differentiation to regenerate pigmented hair (Lu et al., 2020; Rabbani et al., 2011). During anagen, differentiated McSC progeny located in the hair bulb produce and transfer pigment to adjacent epithelial cells that differentiate into the hair. According to Sharov et al (Sharov et al., 2005), at the beginning of telogen, differentiated melanocytes undergo apoptosis in sync with degeneration of the lower part of the HF. Upon entry into the new anagen phase, EpSCs regenerate the lower follicle while McSCs repopulate the hair bulb with differentiated pigment-producing progeny. Thus, these two stem cell

populations act in concert to regenerate pigmented hair with each hair cycle. Several studies have identified numerous signaling inputs received by EpSCs and McSCs (Lu et al., 2020; Rabbani et al., 2011; Zhang et al., 2020) and among them, Wnt signaling remains a key pathway that couples the behavior of these two stem cells. Rabbani et al (Rabbani et al., 2011), adopted genetic mouse models that specifically target either EpSCs or McSCs and showed that Wnt activation in McSCs drives their differentiation into pigment-producing melanocytes, while EpSCs Wnt signaling dictates hair follicle formation and regulates McSCs proliferation during hair regeneration. Thus, Wnt signaling in McSCs is critical for McSC differentiation and hair pigmentation and consistent with the previously proposed theory (Inomata et al., 2009) that the untimely differentiation of McSCs eventually leads to their decreased numbers in the bulge. However, stress can impact hair pigmentation, although it is still debatable whether loss of pigmentation is genetically pre-programmed, or stress-induced. Recently, Zhang et al (Zhang et al., 2020) speculated that stress-induced McSCs loss could be a possible cause of accelerated aging and hair graying. In addition to Wnt signaling, other signaling mechanisms and pathways in EpSCs may influence McSCs and may serve as additional means of communication between these two cell types. Further detailed investigation of additional signaling pathways may add to other types of communication between these distinct stem cell populations.

HFs undergo cyclical spells including telogen, anagen, and catagen. During telogen, hair follicle stem cells (HFSCs) reside in quiescence at the HF bulge. Dermal papilla (DP), residing just beneath the telogen bulge establishes stimulatory molecular crosstalk with neighboring HFSCs and prepares the roadmap of events in anagen. Then HFSCs begin to divide upon overriding quiescence factors to form an outer root sheath. The HFSCs' niche and coordinating episodes have been extensively summarized and reviewed elsewhere (Fuchs and Blau, 2020). In mammals, aged skin showed a significant reduction in HFSCs numbersthrough

differentiation, changing to an epidermal fate during aging, or simply lost. To evaluate the impact of aging on HFSCs, Ge et al. adopted single-cell RNA sequencing to interrogate aging-related changes in HFSCs, and found a decline in their numbers without showing any signs of shifting to an epidermal fate (Ge et al., 2020). These authors observed marked age-related changes in many nonepithelial cell types, including resident immune cells, sensory neurons, and arrector pili muscle cells. Genetic lesions caused by endogenous or exogenous stress impact the survival and function of stem cells. Throughout their lifecycle, these cells experience numerous genotoxic insults from endogenous metabolic byproducts of ROS and inflammation and exogenous environment pollutants and radiation, which can damage DNA (Yang et al., 2022). The DNA damage can cause proteolysis of COL17A1 in aging HFSCs and lead to HFSC transepidermal elimination (Matsumura et al., 2016). It has been shown that physical and/or genotoxic damages cause miR-31 upregulation in aging HFSCs. The upregulated miR-31 activate mitogen-activated protein kinases (MAPK) and drives HFSC transepidermal elimination. While blocking the HFSC transepidermal elimination by COL17A1 overexpression, miR-31 ablation or MAPK/ERK inhibition can significantly slow down HF aging in mice (Matsumura et al., 2016; Morinaga et al., 2021). This can be interpreted that HF aging is not just wear and tear, but a programmed and targetable process.

Mechanistic (originally “mammalian”) Target of Rapamycin Complex 1 (mTORC1) is a multiprotein complex that regulates multiple cellular processes including proliferation, autophagy, and Wnt signaling. mTOR is a highly conserved serine/threonine kinase that interacts with proteins to form two independent complexes: mTORC1 and mTORC2. mTORC1 signaling regulates the behavior of HFSCs (Castilho et al., 2009). The activity of mTORC1 has been associated with HF pigmentation and it has been shown that inhibition of mTORC1 with Rapa in organ-cultured, healthy human scalp anagen HFs, caused up-regulation of hair matrix keratinocyte proliferation, prolonged anagen, and downregulation of apoptosis

(Cheret et al., 2021). This can be interpreted as mTORC1 controling HF pigmentation and growth. According to Nanba et al (Nanba et al.), mTOR remains a key modulator of the response of HFSCs to a variety of physical or chemical signals (temperature, microwave, pH, hypoxia, varying nutrient levels and growth factors).

Air-borne pollutants, such as nitric oxides, carbon monoxide, volatile organic compounds, and particulate matter, get deposited on the scalp and HF, further inducing oxidative stress (Rajput, 2015). According to Mosley and Gibbs (Mosley and Gibbs, 1996), smoking induces premature skin aging, graying of hair, and loss of hair. As reviewed by Trüeb (Trüeb, 2003), the mechanism by which smoking causes hair loss is multifactorial and mostly related to consequences of cigarette smoke on the microvasculature of the dermal hair papilla, damage to DNA of the HF, imbalance in the follicular protease/antiprotease systems, and release of pro-inflammatory cytokines resulting in follicular microinflammation and perifollicular fibrosis. The scalp commensal organism, *Malassezia*, has been recognized as a source of oxidative damage (Trüeb et al., 2018a).The accumulated literature has demonstrated hair protein loss due to UVB radiation and color change by UVA, while high and/or low UVA+UVB doses induced oxidative DNA damage and cytotoxicity in human hair follicles (Gherardini et al., 2019). The loss of the hair proteins could be due to the absorption of radiations and photochemical degradation of the photosensitive amino acids. According to Mahé et al. (Mahé et al., 2000), physicochemical stress from UVR to keratinocytes causes the production of ROS and the release of proinflammatory cytokines causing injury to follicular stem cells of the hair follicle. In summary, environmental pollutants including cigarette smoke, nitric oxides, volatile organic compounds, particulate matter, and ultraviolet radiation play a critical role in ROS production which causes damage to HS, HF, pigmentary unit etc.

Polycyclic aromatic hydrocarbons (PAH), generated by incomplete combustion of organic matter and discharged as environmental contaminants from various activities, such as

transport, wood combustion, coal-fired power plants, or tobacco smoke, damage the hair upon long-term exposure (Palazzi et al., 2018). The hair-cortex degradation and cuticle delamination was enhanced with exposure to increased PAH concentrations (Naudin et al., 2019). Hair exposure to physiological concentrations of PAH and UV irradiation triggers a detrimental effect on the hair microstructure, initiates photoaging of the hair, and generates oxidative stress (Møller et al., 2014; Naudin et al., 2019). In summary, the oxidative stress induced by UV light, psychoemotional, inflammatory, oxidized scalp lipids (Akar et al., 2002), an imbalance of scalp commensal organisms (Trüeb et al., 2018a), and other pollutants contribute to endogenous melanogenic stress, leading to an imbalance between ROS and antioxidant activities (Fig. 4); with increasing age, the production of free radicals increases while the endogenous defense mechanisms weaken, leading to progressive cellular and structural damage and the aging phenotype.

5. MAJOR OPEN QUESTIONS

The HF provides the nutrients for HS growth through its exposure to an internal metabolic environment such as circulating blood, extracellular fluids, and the lymphatic system. Micronutrients maintain intermediary metabolism, and play crucial roles as co-factors or co-enzymes in virtually all enzymatic and hormonal activities, and they also neutralize the deleterious effects of oxidant species, etc. The consequences of their absence are thus severe. Iodine, vitamin A and iron are the most important micronutrients in maintaining public health; their lack represents a major threat to health and development globally, particularly in children and pregnant women. Any imbalances or deficiencies of these nutrients can trigger a chain reaction resulting in illness, undernourishment, and accumulation of toxic elements. Recent literature concludes that the HS retains different molecules, metabolites, therapeutic drugs, heavy metals and other environmental pollutants. Hair grows about 1 cm per month, thus,

analysis of a typical 2 cm segment of the HS close to the scalp may provide at least two month's record of metabolic activities, mineral deficiencies, metals toxicity and exposure to environmental pollutants in an individual. Retrieving such records from HS can revolutionize the medical field in monitoring health, nutrient deficiencies, and toxin levels. Further, such an approach may help in forensic sciences to retrieve drug-related history, drug abuse, and geolocation of a suspect through environmental exposure. Hair tissue mineral analysis (HTMA) could be a valuable approach since it measures the mineral content of a hair sample and informs proper intervention through diet design and supplements to fit an individual's specific needs. However, literature on hair tissue mineral analysis (HTMA) is scarce, and very limited efforts have been invested. This raises an intriguing prospect of designing and executing future research based on such an approach, which has been neglected so far. To support this, the question remains whether advanced, sensitive, and validated methodologies to retrieve HF-stored information can be developed effectively and in a timely manner. If HTMA is well-developed, it may provide key information about the human body's nutritional status, health, lifestyle, toxin accumulation etc. Hair samples can therefore be effective specimens for monitoring health status and exposure to toxins, and the preferred sample type over blood or urine which are harder to collect and provide only short-term information.

6. CHALLENGES AND FUTURE PERSPECTIVES

Aging is a natural and complex biological process that is influenced not only by an individual's intrinsic determinants of genomics, transcriptomics, proteomics, and metabolomics but also by extrinsic factors such as lifestyle, diet, and environment. Recent advances in "omics" technologies, including genome sequencing, quantitative protein expression analysis, site-specific post-translational protein modification analysis, and metabolite quantification, have contributed to innovative breakthroughs in our understanding

of biological processes and disease mechanisms and the discovery of diagnostic biomarkers and therapeutics. These techniques offer high throughput, sensitivity, better resolution, and rapid scanning speed. Omics technologies have been adopted in drug detection, protein, lipid, and metabolite analysis, and the discovery of different protein modifications.

HS profiling by "omics" technologies is well enlightened by recent applications in personalized medicine, including HIV infection, tuberculosis, chronic psychosocial stress, myocardial infarction, chronic alcohol abuse, diabetes mellitus, drug abuse, environmental toxins, neurodegenerative diseases, and cancers (Ademola Adeola et al., 2018). Hair assessment by omics methodologies could be further extended to other diseases to discover respective disease biomarkers. Blood or urine provides short-term information related to drug addiction, while HS analysis provides long-term history because drugs incorporated in the hair are very stable over time. The scalp hair grows longer, and depending on the length, both endogenous and exogenous metabolites can be monitored over an extended period, which could be useful in monitoring disease progression and delivering optimum patient care.

Mass spectrometry approaches have been successfully exploited in hair analysis and established potential in quantitative profiling of more than 300 proteins, biomarkers of oxidative damage, aging, structural damage, hair diseases like monilethrix, alopecia, and drugs, including cocaine. The proteomics database is well established, but establishing the hair metabolome remains the major challenge due to the lack of a well-established, comprehensive, electronically accessible database. Rendering to human metabolome database 4.0 (HMDB 4.0), the total number of metabolites is 114,100, of which 18,609 can be detected and quantified (https://hmdb.ca/statistics#biospecimen-statistics) (Wishart et al., 2018). The human metabolome database covers metabolic biomarkers from blood, feces, and urine, but hair samples have not yet been given much attention as biological specimens. Mass spectrometric

methodologies have been adopted in hair analysis in the past decade, but limited omics literature exists. Subsequent studies should aim to fill this information gap. Active therapeutics are often applied topically through hair follicular translocation routes. Advancing the technologies and validating methodologies in monitoring the efficacy of the therapeutics, drug-protein interactions, signaling mechanisms, and hair-related disorders may bring innovations in hair care and cosmetics.

Hair metabolites that alter with age are inconclusive, and the main questions in hair aging research remain unanswered. First, the complex interactions of proteins, regulatory networks, signaling pathways, and altered metabolic pathways need to be established to understand the complex process of hair aging and identify the hair aging biomarkers. Changes in hair structure either due to disease or dynamic responses to certain environmental or growth conditions are being observed through histopathology, histo-immunological imaging, SEM, TEM and other microscopy techniques. Recent advances in direct electron detectors, ultrastable electron microscopes and high-resolution cryo-EM have increased the throughput of structural biology and macromoleular studies. More recently, advances in single-particle cryogenic electron microscopy (cryo-EM) have opened up interesting new opportunities to complement omics approaches with structural biology analyses. The high-resolution structures of a proteome acquired with these methods, in combination with its spatial context obtained with electron tomography, will produce information-rich cell atlases that can be synergized with other omics approaches (Fig 5). The integration of data from multi-omics platforms and structural biology (immunohistonorphometry and microscopy) may provide a better understanding of the structure-function relationship and the biological mechanism of HF aging. However, this integration of multi-omics data is challenging due to the complexity of annotation, mapping with pathways, network analysis, interactions and the practical difficulty of differentiating biological aging from aging due to environmental factors.

Structural analyses of HS and HF have been conducted via light microscopy combined with silver impregnation or immunohistochemistry, TEM and SEM (Kaidoh and Inoué, 2000; Takahashi-Iwanaga, 2000). HF is an architecturally and functionally complex organ. HFs are innervated by palisade nerve endings (PNs) such as stockade and perifollicular nerve endings, circumferential (pilo-Ruffini) nerve endings, and Merkel nerve endings (Furuta et al., 2020; Ghitani et al., 2017). The PNs are composed of lanceolate nerve endings (LNEs) and are situated at the isthmus. LNEs of terminal HFs in the human scalp were successfully analyzed using light-sheet microscopy and correlative light and electron microscopy (CLEM), a combination of confocal laser microscopy and TEM (Yamanishi and Iwabuchi, 2023). In fact, these authors adopted 3D-CLEM, a combination of confocal laser microscopy and focused ion beam scanning electron microscopy (FIB–SEM) as well as immunofluorescence technology to understand the 3D ultrastructure of LNEs. Such ultrastructural data of each component of HSs and HFs obtained via combinations of microscopy, immonohistomorphometry, and quantitative omics data (genomics, transcriptomics, proteomics, and metabolomics) need to be integrated to gain complete understanding of the function and regulatory role of each biomolecule. While the possibilities are clear, the main bottleneck currently remains the lack of adequate data since proteomics techniques are just a decade old and metabolomics is still in the developmental stage. Protein interactions identified through mass spectrometry in conjunction with advances in 3D structure could be very useful in investigating architectural changes in HS and HF during development, aging and pathology. This requires systematic evaluation of observed alterations and differentiation between normal age-associated changes and changes due to environmental exposure and lifestyle, i.e., biological aging vs. environmental aging. The challenge remains the integration of data from the various platforms, which will require researchers to overcome complications associated with annotation, pathway mapping, and networking.

Integrating structural morphological data with genomics, proteomics, and metabolomics data of only single cell types, such as melanocyte or DPC, may be less complicated. Doing that for each cell component of HS and HF during aging or growth cycle with other omics data via multiple bioinformatic platforms would provide insights into hair aging and hair graying. However, it needs systematic computational pipelines for sorting, identifying, and performing molecular modeling of the myriad of structures. Moreover, the potential cell sources are still poor due to cell aging within *in vitro* culture systems and inefficient cell reprogramming, hence, significant efforts are required to develop and improve advance *in vitro* culture systems. Significant efforts are being employed in HF regeneration and recent advances and challenges are reviewed elsewhere (Ji et al., 2021). The mechanism of hair cycling is very complex, and it is extremely difficult to identify key molecules involved in the process. However, an integrated approach of structural data obtained via immunohistomorphometry, electron microscopy (cryo-EM) and different microscopy; together with multi-omics data acquired through genomics, transcriptomics, proteomics, and metabolomics may provide an in-depth understanding of the key molecules that control and regulate HS and HF functions. Finally, morphological and multi-omics data collected during the transition of black-to-gray hair, in comparison to data obtained from completely black or gray hair may provide key insights into the mechanisms of hair graying.

Conflicts of interest

The authors declare no competing interests of any kind.

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6. REFERENCES

Adav, S.S., Gallart-Palau, X., Tan, K.H., Lim, S.K., Tam, J.P., Sze, S.K., 2016. Dementia-linked amyloidosis is associated with brain protein deamidation as revealed by proteomic profiling of human brain tissues. Mol. Brain 9, 20.

Adav, S.S., Hwa, H.H., de Kleijn, D., Sze, S.K., 2015. Improving blood plasma glycoproteome coverage by coupling ultracentrifugation fractionation to electrostatic repulsion–hydrophilic interaction chromatography enrichment. J. Proteome Res. 14, 2828-2838.

Adav, S.S., Park, J.E., Sze, S.K., 2019. Quantitative profiling brain proteomes revealed mitochondrial dysfunction in alzheimer's disease. Mol. Brain 12, 8.

Adav, S.S., Subbaiaih, R.S., Kerk, S.K., Lee, A.Y., Lai, H.Y., Ng, K.W., Sze, S.K., Schmidtchen, A., 2018. Studies on the proteome of human hair - identification of histones and deamidated keratins. Sci. Rep. 8, 1599.

Adav, S.S., Sze, S.K., 2020. Hypoxia-induced degenerative protein modifications associated with aging and age-associated disorders. Aging Dis. 11, 341.

Adav, S.S., Wang, Y., 2021. Metabolomics signatures of aging: Recent advances. Aging Dis. 12, 646.

Ademola Adeola, H., Cathryn Van Wyk, J., Temitope Arowolo, A., Patience Khumalo, N., 2018. Human hair as a testing substrate in the era of precision medicine: Potential role of 'omics-based approaches, Keratin. IntechOpen.

Adhikari, K., Fontanil, T., Cal, S., Mendoza-Revilla, J., Fuentes-Guajardo, M., Chacón-Duque, J.-C., Al-Saadi, F., Johansson, J.A., Quinto-Sanchez, M., Acuña-Alonzo, V., 2016. A genome-wide association scan in admixed latin americans identifies loci influencing facial and scalp hair features. Nat. Commun. 7, 10815.

Agaoglu, E., Kaya Erdogan, H., Acer, E., Atay, E., Metintas, S., Saracoglu, Z.N., 2021. Prevalence of early-onset androgenetic alopecia and its relationship with lifestyle and dietary habits. Ital J Dermatol Venerol 156, 675-680.

Akar, A., Arca, E., Erbil, H., Akay, C., Sayal, A., Gür, A.R., 2002. Antioxidant enzymes and lipid peroxidation in the scalp of patients with alopecia areata. J. Dermatol. Sci. 29, 85-90.

Al-Nuaimi, Y., Hardman, J.A., Bíró, T., Haslam, I.S., Philpott, M.P., Tóth, B.I., Farjo, N., Farjo, B., Baier, G., Watson, R.E., 2014. A meeting of two chronobiological systems: Circadian proteins period1 and bmal1 modulate the human hair cycle clock. J. Invest. Dermatol. 134, 610-619.

Ambatipudi, S., Horvath, S., Perrier, F., Cuenin, C., Hernandez-Vargas, H., Le Calvez-Kelm, F., Durand, G., Byrnes, G., Ferrari, P., Bouaoun, L., Sklias, A., Chajes, V., Overvad, K., Severi, G., Baglietto, L., Clavel-Chapelon, F., Kaaks, R., Barrdahl, M., Boeing, H., Trichopoulou, A., Lagiou, P., Naska, A., Masala, G., Agnoli, C., Polidoro, S., Tumino, R., Panico, S., Dollé, M., Peeters, P.H.M., Onland-Moret, N.C., Sandanger, T.M., Nøst, T.H., Weiderpass, E., Quirós, J.R., Agudo, A., Rodriguez-Barranco, M., Huerta Castaño, J.M., Barricarte, A., Fernández, A.M., Travis, R.C., Vineis, P., Muller, D.C., Riboli, E., Gunter, M., Romieu, I., Herceg, Z., 2017. DNA methylome analysis identifies accelerated epigenetic ageing associated with postmenopausal breast cancer susceptibility. Eur. J. Cancer 75, 299-307.

Anderson, J.H., Tester, D.J., Will, M.L., Ackerman, M.J., 2016. Whole-exome molecular autopsy after exertion-related sudden unexplained death in the young. Circ. Cardiovasc. Genet. 9, 259-265.

Arck, P.C., Overall, R., Spatz, K., Liezman, C., Handjiski, B., Klapp, B.F., Birch-Machin, M.A., Peters, E.M.J., Arck, P.C., Overall, R., 2006. Towards a "free radical theory of

graying": Melanocyte apoptosis in the aging human hair follicle is an indicator of oxidative stress induced tissue damage. FASEB J. 20, 1567-1569.
Armitage, E.G., Ciborowski, M., 2017. Applications of metabolomics in cancer studies. Adv Exp Med Biol 965, 209-234.
Aviv, A., Shay, J.W., 2018. Reflections on telomere dynamics and ageing-related diseases in humans. Philos. Trans. R. Soc. Lond., B, Biol. Sci. 373, 20160436.
Baker, L.B., 2019. Physiology of sweat gland function: The roles of sweating and sweat composition in human health. Temperature (Austin) 6, 211-259.
Balañá, M.E., Charreau, H.E., Leirós, G.J., 2015. Epidermal stem cells and skin tissue engineering in hair follicle regeneration. World J. Stem Cells 7, 711-727.
Banerjee, D.K., Zhang, Z., Baksi, K., Serrano-Negrón, J.E., 2017. Dolichol phosphate mannose synthase: A glycosyltransferase with unity in molecular diversities. Glycoconjugate J. 34, 467-479.
Bart, G., Fischer, D., Samoylenko, A., Zhyvolozhnyi, A., Stehantsev, P., Miinalainen, I., Kaakinen, M., Nurmi, T., Singh, P., Kosamo, S., Rannaste, L., Viitala, S., Hiltunen, J., Vainio, S.J., 2021. Characterization of nucleic acids from extracellular vesicle-enriched human sweat. BMC Genomics 22, 425.
Barthélemy, N.R., Bednarczyk, A., Schaeffer-Reiss, C., Jullien, D., Van Dorsselaer, A., Cavusoglu, N., 2012. Proteomic tools for the investigation of human hair structural proteins and evidence of weakness sites on hair keratin coil segments. Anal. Biochem. 421, 43-55.
Baumann, L., 2007. Skin ageing and its treatment. The Journal of Pathology 211, 241-251.
Baxter, L.L., Watkins-Chow, D.E., Pavan, W.J., Loftus, S.K., 2019. A curated gene list for expanding the horizons of pigmentation biology. Pigment Cell Melanoma Res 32, 348-358.
Beger, R.D., 2013. A review of applications of metabolomics in cancer. Metabolites 3, 552-574.
Bildstein, L., Deniset-Besseau, A., Pasini, I., Mazilier, C., Keuong, Y.W., Dazzi, A., Baghdadli, N., 2020. Discrete nanoscale distribution of hair lipids fails to provide humidity resistance. Anal. Chem. 92, 11498-11504.
BioMed research internationalOliver, M.A., Marti, M., Coderch, L., Carrer, V., Kreuzer, M., Barba, C., 2019. Lipid loses and barrier function modifications of the brown-to-white hair transition. Skin Res. Technol. 25, 517-525.
Birgersdotter, A., Sandberg, R., Ernberg, I., 2005. Gene expression perturbation in vitro—a growing case for three-dimensional (3d) culture systems, Seminars in cancer biology. Elsevier, pp. 405-412.
Botchkareva, N.V., Botchkarev, V.A., Gilchrest, B.A., 2003. Fate of melanocytes during development of the hair follicle pigmentary unit. J. Investig. Dermatol. Symp. Proc. 8, 76-79.
Bovell, D.L., 2018. The evolution of eccrine sweat gland research towards developing a model for human sweat gland function. Exp. Dermatol. 27, 544-550.
Bovell, D.L., MacDonald, A., Meyer, B.A., Corbett, A.D., MacLaren, W.M., Holmes, S.L., Harker, M., 2011. The secretory clear cell of the eccrine sweat gland as the probable source of excess sweat production in hyperhidrosis. Exp. Dermatol. 20, 1017-1020.
Breloy, I., Hanisch, F.-G., 2018. Functional roles of o-glycosylation. MDPI, p. 3063.
Brosche, T., Dressler, S., Platt, D., 2001. Age-associated changes in integral cholesterol and cholesterol sulfate concentrations in human scalp hair and finger nail clippings. Aging Clin. Exp. Res. 13, 131-138.

Bujak, R., Struck-Lewicka, W., Markuszewski, M.J., Kaliszan, R., 2015. Metabolomics for laboratory diagnostics. Journal of Pharmaceutical and Biomedical Analysis 113, 108-120.
Camera, E., Dahlhoff, M., Ludovici, M., Zouboulis, C.C., Schneider, M.R., 2014. Perilipin 3 modulates specific lipogenic pathways in sz 95 sebocytes. Exp. Dermatol. 23, 759-761.
Carlson, T.R., Hu, H., Braren, R., Kim, Y.H., Wang, R.A., 2008. Cell-autonomous requirement for beta1 integrin in endothelial cell adhesion, migration and survival during angiogenesis in mice. Development 135, 2193-2202.
Carré, J.-L., Suzuki, T., Paus, R., 2020. Do hair follicles operate as primitive, multifocal kidney-like excretory (mini-) organs? Exp. Dermatol. 29, 357-365.
Castilho, R.M., Squarize, C.H., Chodosh, L.A., Williams, B.O., Gutkind, J.S., 2009. Mtor mediates wnt-induced epidermal stem cell exhaustion and aging. Cell stem cell 5, 279-289.
Chaudhuri, J., Bains, Y., Guha, S., Kahn, A., Hall, D., Bose, N., Gugliucci, A., Kapahi, P., 2018. The role of advanced glycation end products in aging and metabolic diseases: Bridging association and causality. Cell Metab. 28, 337-352.
Chen, C.-C., Murray, P.J., Jiang, T.X., Plikus, M.V., Chang, Y.-T., Lee, O.K., Widelitz, R.B., Chuong, C.-M., 2014. Regenerative hair waves in aging mice and extra-follicular modulators follistatin, dkk1, and sfrp4. J. Invest. Dermatol. 134, 2086-2096.
Chen, J., Fan, Z.-X., Zhu, D.-C., Guo, Y.-L., Ye, K., Dai, D., Guo, Z., Hu, Z.-Q., Miao, Y., Qu, Q., 2021. Emerging role of dermal white adipose tissue in modulating hair follicle development during aging. Front. Cell Dev. Biol. 9.
Cheret, J., Suzuki, T., Scala, F., O'Sullivan, J., Nicu, C., Gherardini, J., Epstein-Kuka, G., Bauman, A., Demetriades, C., Paus, R., 2021. 602 mtorc1 activity controls human scalp hair follicle pigmentation and growth. J. Invest. Dermatol. 141, S104.
Choi, Y.J., Yoon, T.J., Lee, Y.H., 2008. Changing expression of the genes related to human hair graying. Eur. J. Dermatol. 18, 397-399.
Chojnacka, K., Górecka, H., Górecki, H., 2006. The effect of age, sex, smoking habit and hair color on the composition of hair. Environmental Toxicology and Pharmacology 22, 52-57.
Chu, F., Mason, K.E., Anex, D.S., Jones, A.D., Hart, B.R., 2020. Proteomic characterization of damaged single hairs recovered after an explosion for protein-based human identification. J. Proteome Res. 19, 3088-3099.
Chua, H.M., Zhao, Z., Ng, K.W., 2020. Cryogelation of human hair keratins. Macromol. Rapid Commun. 41, 2000254.
Claffey, D.J., Stout, P.R., Ruth, J.A., 2001. 3h-nicotine, 3h-flunitrazepam, and 3h-cocaine incorporation into melanin: A model for the examination of drug-melanin interactions. J. Anal. Toxicol. 25, 607-611.
Cloos, P.A.C., Christgau, S., 2004. Post-translational modifications of proteins: Implications for aging, antigen recognition, and autoimmunity. Biogerontology 5, 139-158.
Clunes, M., Lindsay, S., Roussa, E., Quinton, P., Bovell, D., 2004. Localisation of the vacuolar proton pump (vh+-atpase) and carbonic anhydrase ii in the human eccrine sweat gland. J. Mol. Histol. 35, 339-345.
Coderch, L., Lorenzo, R.D., Mussone, M., Alonso, C., Martí, M., 2022. The role of lipids in the process of hair ageing. Cosmetics 9.
Coderch, L., Oliver, M.A., Martínez, V., Manich, A.M., Rubio, L., Martí, M., 2017. Exogenous and endogenous lipids of human hair. Skin Res. Technol. 23, 479-485.
Cohen, J., 1961. The transplantation of individual rat and guinea-pig whisker papillae.
Commo, S., Bernard, B.A., 2000. Melanocyte subpopulation turnover during the human hair cycle: An immunohistochemical study. Pigment Cell Res. 13, 253-259.

Commo, S., Gaillard, O., Bernard, B.A., 2004. Human hair greying is linked to a specific depletion of hair follicle melanocytes affecting both the bulb and the outer root sheath. Br. J. Dermatol. 150, 435-443.
Cornellison, C., Dyer, J., Plowman, J., Krsinic, G., Clerens, S., 2011. Maldi-ms redox lipidomics applied to human hair: A first look. Int. J. Trichology 3, 25-27.
Costin, G.E., Hearing, V.J., 2007. Human skin pigmentation: Melanocytes modulate skin color in response to stress. FASEB J. 21, 976-994.
Crunelle, C.L., Yegles, M., van Nuijs, A.L., Covaci, A., De Doncker, M., Maudens, K.E., Sabbe, B., Dom, G., Lambert, W.E., Michielsen, P., 2014. Hair ethyl glucuronide levels as a marker for alcohol use and abuse: A review of the current state of the art. Drug Alcohol Depend. 134, 1-11.
Cruz, C., Della Rosa, M., Krueger, C., Gao, Q., Horkai, D., King, M., Field, L., Houseley, J., 2018. Tri-methylation of histone h3 lysine 4 facilitates gene expression in ageing cells. Elife 7, 10.7554/eLife.34081.
Cruz, C.F., Fernandes, M.M., Gomes, A.C., Coderch, L., Martí, M., Méndez, S., Gales, L., Azoia, N.G., Shimanovich, U., Cavaco-Paulo, A., 2013. Keratins and lipids in ethnic hair. Int. J. Cosmetic Sci. 35, 244-249.
Csuka, D.A., Csuka, E.A., Juhász, M.L.W., Sharma, A.N., Mesinkovska, N.A., 2023. A systematic review on the lipid composition of human hair. Int. J. Dermatol. 62, 404-415.
D'Mello, S.A.N., Finlay, G.J., Baguley, B.C., Askarian-Amiri, M.E., 2016. Signaling pathways in melanogenesis. International Journal of Molecular Sciences 17, 1144.
Dahlhoff, M., Camera, E., Picardo, M., Zouboulis, C.C., Chan, L., Chang, B.H.-J., Schneider, M.R., 2013. Plin2, the major perilipin regulated during sebocyte differentiation, controls sebaceous lipid accumulation in vitro and sebaceous gland size in vivo. Biochimica et Biophysica Acta (BBA)-General Subjects 1830, 4642-4649.
Dahlhoff, M., Fröhlich, T., Arnold, G.J., Müller, U., Leonhardt, H., Zouboulis, C.C., Schneider, M.R., 2015. Characterization of the sebocyte lipid droplet proteome reveals novel potential regulators of sebaceous lipogenesis. Exp. Cell Res. 332, 146-155.
Davidovic, M., Sevo, G., Svorcan, P., Milosevic, D.P., Despotovic, N., Erceg, P., 2010. Old age as a privilege of the "selfish ones". Aging Dis 1, 139-146.
Dawber, R., 1996. Hair: Its structure and response to cosmetic preparations. Clin. Dermatol. 14, 105-112.
de Seymour, J.V., Tu, S., He, X., Zhang, H., Han, T.L., Baker, P.N., Sulek, K., 2018. Metabolomic profiling of maternal hair suggests rapid development of intrahepatic cholestasis of pregnancy. Metabolomics 14, 79.
De Tollenaere, M., Chapuis, E., Auriol, P., Auriol, D., Scandolera, A., Reynaud, R., 2021. Global repigmentation strategy of grey hair follicles by targeting oxidative stress and stem cells protection. Applied Sciences 11, 1533.
Diz, A.P., Martinez-Fernandez, M., Rolan-Alvarez, E., 2012. Proteomics in evolutionary ecology: Linking the genotype with the phenotype. Molecular Ecology 21, 1060-1080.
Doolittle, J., Walker, P., Mills, T., Thurston, J., 2016. Hyperhidrosis: An update on prevalence and severity in the united states. Arch. Dermatol. Res. 308, 743-749.
Duong, V.-A., Park, J.-M., Lim, H.-J., Lee, H., 2021. Proteomics in forensic analysis: Applications for human samples. Applied Sciences 11, 3393.
Eisenbeiss, L., Binz, T.M., Baumgartner, M.R., Kraemer, T., Steuer, A.E., 2020a. Cheating on forensic hair testing? Detection of potential biomarkers for cosmetically altered hair samples using untargeted hair metabolomics. Analyst 145, 6586-6599.

Eisenbeiss, L., Binz, T.M., Baumgartner, M.R., Kraemer, T., Steuer, A.E., 2020b. Towards best practice in hair metabolomic studies: Systematic investigation on the impact of hair length and color. Metabolites 10.
ElFaramawy, A.A.A., Hanna, I.S., Darweesh, R.M., Ismail, A.S., Kandil, H.I., 2018. The degree of hair graying as an independent risk marker for coronary artery disease, a ct coronary angiography study. Egypt. Heart J. 70, 15-19.
Elliott, K., Stephenson, T.J., Messenger, A.G., 1999. Differences in hair follicle dermal papilla volume are due to extracellular matrix volume and cell number: Implications for the control of hair follicle size and androgen responses. J Invest Dermatol 113, 873-877.
Erdoğan, B., 2017. Anatomy and physiology of hair, hair and scalp disorders, zekayi kutlubay and server serdaroglu, intechopen, doi: 10.5772/67269.
Ezure, T., Amano, S., 2012. Involvement of upper cheek sagging in nasolabial fold formation. Skin Res. Technol. 18, 259-264.
Ezure, T., Amano, S., Matsuzaki, K., 2022. Infiltration of subcutaneous adipose layer into the dermal layer with aging. Skin Res. Technol. 28, 311-316.
Fernandes Durso, D., Giulia Bacalini, M., Sala, C., Pirazzini, C., Marasco, E., Bonafé, M., do Valle, Í.F., Gentilini, D., Castellani, G., Caetano Faria, A.M., Franceschi, C., Garagnani, P., Nardini, C., 2017. Acceleration of leukocytes' epigenetic age as an early tumor and sex-specific marker of breast and colorectal cancer. Oncotarget 8: 23237-23245. doi: 10.18632/oncotarget.15573.
Fernandez-Flores, A., Saeb-Lima, M., Cassarino, D.S., 2019. Histopathology of aging of the hair follicle. J. Cutan. Pathol. 46, 508-519.
Festa, E., Fretz, J., Berry, R., Schmidt, B., Rodeheffer, M., Horowitz, M., Horsley, V., 2011. Adipocyte lineage cells contribute to the skin stem cell niche to drive hair cycling. Cell 146, 761-771.
Field, A.E., Robertson, N.A., Wang, T., Havas, A., Ideker, T., Adams, P.D., 2018. DNA methylation clocks in aging: Categories, causes, and consequences. Mol. Cell 71, 882-895.
Fischer, T.W., Slominski, A., Tobin, D.J., Paus, R., 2008. Melatonin and the hair follicle. J. Pineal Res. 44, 1-15.
Fousteri, M., Mullenders, L.H., 2008. Transcription-coupled nucleotide excision repair in mammalian cells: Molecular mechanisms and biological effects. Cell research 18, 73-84.
Franklin, R.N., Karim, N., Goecker, Z.C., Durbin-Johnson, B.P., Rice, R.H., Parker, G.J., 2020. Proteomic genotyping: Using mass spectrometry to infer snp genotypes in pigmented and non-pigmented hair. Forensic Sci. Int. 310, 110200.
Franzka, P., Krüger, L., Schurig, M.K., Olecka, M., Hoffmann, S., Blanchard, V., Hübner, C.A., 2021. Altered glycosylation in the aging heart. Front. Mol. Biosci. 8.
Fuchs, E., Blau, H.M., 2020. Tissue stem cells: Architects of their niches. Cell Stem Cell 27, 532-556.
Fujioka, Y., Kimata, Y., Nomaguchi, K., Watanabe, K., Kohno, K., 2002. Identification of a novel non-structural maintenance of chromosomes (smc) component of the smc5-smc6 complex involved in DNA repair. J. Biol. Chem. 277, 21585-21591.
Furuta, T., Bush, N.E., Yang, A.E., Ebara, S., Miyazaki, N., Murata, K., Hirai, D., Shibata, K.I., Hartmann, M.J.Z., 2020. The cellular and mechanical basis for response characteristics of identified primary afferents in the rat vibrissal system. Curr Biol 30, 815-826.e815.
Gandhi, M., Ameli, N., Bacchetti, P., Gange, S.J., Anastos, K., Levine, A., Hyman, C.L., Cohen, M., Young, M., Huang, Y., 2009. Protease inhibitor levels in hair samples strongly predict virologic responses to hiv treatment. AIDS (London, England) 23, 471.

Ge, Y., Miao, Y., Gur-Cohen, S., Gomez, N., Yang, H., Nikolova, M., Polak, L., Hu, Y., Verma, A., Elemento, O., Krueger, J.G., Fuchs, E., 2020. The aging skin microenvironment dictates stem cell behavior. Proc. Natl. Acad. Sci. U. S. A. 117, 5339-5350.

Gentile, P., De Giglio, E., Hilborn, J.G., Ng, K.W., 2021. Editorial: Composite and functionalized hydrogels: Implications for improved and biological properties in tissue engineering. Front. Bioeng. Biotechnol. 8, 636575-636575.

Gerona, R., Wen, A., Chin, A.T., Koss, C.A., Bacchetti, P., Metcalfe, J., Gandhi, M., 2016. Quantifying isoniazid levels in small hair samples: A novel method for assessing adherence during the treatment of latent and active tuberculosis. PLoS One 11, e0155887.

Gherardini, J., Wegner, J., Chéret, J., Ghatak, S., Lehmann, J., Alam, M., Jimenez, F., Funk, W., Böhm, M., Botchkareva, N.V., Ward, C., Paus, R., Bertolini, M., 2019. Transepidermal uv radiation of scalp skin ex vivo induces hair follicle damage that is alleviated by the topical treatment with caffeine. Int. J. Cosmet. Sci. 41, 164-182.

Ghitani, N., Barik, A., Szczot, M., Thompson, J.H., Li, C., Le Pichon, C.E., Krashes, M.J., Chesler, A.T., 2017. Specialized mechanosensory nociceptors mediating rapid responses to hair pull. Neuron 95, 944-954.e944.

Giesen, M., Gruedl, S., Holtkoetter, O., Fuhrmann, G., Koerner, A., Petersohn, D., 2011. Ageing processes influence keratin and kap expression in human hair follicles. Exp Dermatol 20, 759-761.

GVR, 2018. Hair care market growth & trends, grand view research. Https://www.Grandviewresearch.Com/industry-analysis/hair-care-market.

Hains, P.G., Truscott, R.J., 2010. Age-dependent deamidation of lifelong proteins in the human lens. Invest. Ophthalmol. Vis. Sci. 51, 3107-3114.

Hao, P., Adav, S.S., Gallart-Palau, X., Sze, S.K., 2017. Recent advances in mass spectrometric analysis of protein deamidation. Mass Spectrom. Rev. 36, 677-692.

Hao, T., Guo, J., Liu, J., Wang, J., Liu, Z., Cheng, X., Li, J., Ren, J., Li, Z., Yan, J., Zhang, G., 2021. Predicting human age by detecting DNA methylation status in hair. ELECTROPHORESIS 42, 1255-1261.

Hardman, J.A., Tobin, D.J., Haslam, I.S., Farjo, N., Farjo, B., Al-Nuaimi, Y., Grimaldi, B., Paus, R., 2015. The peripheral clock regulates human pigmentation. J. Invest. Dermatol. 135, 1053-1064.

Hariri, H., Kurban, M., Al-Haddad, C., Fahed, A.C., Poladian, S., Khalil, A., Abbas, O., Arabi, M., Bitar, F., Nemer, G., 2018. Degenerated hair follicle cells and partial loss of sebaceous and eccrine glands in a familial case of axenfeld-rieger syndrome: An emerging role for the foxc1/nfatc1 genetic axis. J. Dermatol. Sci. 92, 237-244.

Harman, D., 1956. Aging-a theory based on free radical and radiation chemistry. J. Gerontol. 11, 298-300.

Hayakawa, K., Esposito, E., Wang, X., Terasaki, Y., Liu, Y., Xing, C., Ji, X., Lo, E.H., 2016. Transfer of mitochondria from astrocytes to neurons after stroke. Nature 535, 551-555.

Hegedus, Z.L., 2000. The probable involvement of soluble and deposited melanins, their intermediates and the reactive oxygen side-products in human diseases and aging. Toxicology 145, 85-101.

Higgins, C.A., Chen, J.C., Cerise, J.E., Jahoda, C.A.B., Christiano, A.M., 2013. Microenvironmental reprogramming by three-dimensional culture enables dermal papilla cells to induce de novo human hair-follicle growth. Proceedings of the National Academy of Sciences 110, 19679-19688.

Higgins, C.A., Richardson, G.D., Ferdinando, D., Westgate, G.E., Jahoda, C.A., 2010. Modelling the hair follicle dermal papilla using spheroid cell cultures. Experimental dermatology 19, 546-548.
Hough, K.P., Trevor, J.L., Strenkowski, J.G., Wang, Y., Chacko, B.K., Tousif, S., Chanda, D., Steele, C., Antony, V.B., Dokland, T., 2018. Exosomal transfer of mitochondria from airway myeloid-derived regulatory cells to t cells. Redox biology 18, 54-64.
Hsia, C.-W., Shui, H.-A., Wang, C.-Y., Yu, H.-M., Ho, M.-Y., Cheng, K.-T., Tseng, M.-J., 2011. Proteomics demonstration that histone h4 is a colchicine-induced retro-modulator of growth and alkaline phosphatase activity in hair follicle dermal papilla culture. J. Proteomics 74, 805-816.
Imbert, I., Guerif-Ferreira, Y., Oberto, G., Berghi, A., Mouser, P., Cucumel, K., Domloge, N., 2012. In vivo study of age-related skin changes using in vivo confocal microscopy. IFSCC Magazine 1, 15-21.
Inomata, K., Aoto, T., Binh, N.T., Okamoto, N., Tanimura, S., Wakayama, T., Iseki, S., Hara, E., Masunaga, T., Shimizu, H., 2009. Genotoxic stress abrogates renewal of melanocyte stem cells by triggering their differentiation. Cell 137, 1088-1099.
Ito, S., Wakamatsu, K., 2011. Diversity of human hair pigmentation as studied by chemical analysis of eumelanin and pheomelanin. J. Eur. Acad. Dermatol. Venereol. 25, 1369-1380.
Itou, T., Ito, S., Wakamatsu, K., 2019. Effects of aging on hair color, melanosome morphology, and melanin composition in japanese females. Int J Mol Sci 20.
Jahoda, C.A., Reynolds, A.J., 2001. Hair follicle dermal sheath cells: Unsung participants in wound healing. The Lancet 358, 1445-1448.
Jan, H., Wei, M., Peng, C., Lin, S., Lai, P., Shieh, M., 2012. The use of polyethylenimine–DNA to topically deliver htert to promote hair growth. Gene Ther. 19, 86-93.
Jang, W.J., Choi, J.Y., Park, B., Seo, J.H., Seo, Y.H., Lee, S., Jeong, C.H., Lee, S., 2019. Hair metabolomics in animal studies and clinical settings. Molecules 24.
Ji, J.H., Park, T.-S., Lee, H.-J., Kim, Y.-D., Pi, L.-Q., Jin, X.-H., Lee, W.-S., 2013. The ethnic differences of the damage of hair and integral hair lipid after ultra violet radiation. Ann. Dermatol. 25, 54-60.
Ji, S., Zhu, Z., Sun, X., Fu, X., 2021. Functional hair follicle regeneration: An updated review. Signal Transduct. Target Ther. 6, 66.
Jiménez-Cervantes, C., Martínez-Esparza, M., Pérez, C., Daum, N., Solano, F., García-Borrón, J.C., 2001. Inhibition of melanogenesis in response to oxidative stress: Transient downregulation of melanocyte differentiation markers and possible involvement of microphthalmia transcription factor. J. Cell Sci. 114, 2335-2344.
Jin, K., 2010. Modern biological theories of aging. Aging Dis. 1, 72.
Jo, S.J., Kim, J.Y., Jang, S., Choi, S.J., Kim, K.H., Kwon, O., 2016. Decrease of versican levels in the follicular dermal papilla is a remarkable aging-associated change of human hair follicles. J. Dermatol. Sci. 84, 354-357.
Johnson, A.A., Akman, K., Calimport, S.R.G., Wuttke, D., Stolzing, A., de Magalhães, J.P., 2012. The role of DNA methylation in aging, rejuvenation, and age-related disease. Rejuvenation Res. 15, 483-494.
Joo, K.-M., Kim, A.-R., Kim, S.-N., Kim, B.-M., Lee, H.K., Bae, S., Lee, J.-H., Lim, K.-M., 2016. Metabolomic analysis of amino acids and lipids in human hair altered by dyeing, perming and bleaching. Exp. Dermatol. 25, 729-731.
Journal of Proteome ResearchPośpiech, E., Kukla-Bartoszek, M., Karłowska-Pik, J., Zieliński, P., Woźniak, A., Boroń, M., Dąbrowski, M., Zubańska, M., Jarosz, A., Grzybowski, T., Płoski, R., Spólnicka, M., Branicki, W., 2020. Exploring the possibility of predicting

human head hair greying from DNA using whole-exome and targeted ngs data. BMC Genomics 21, 538.
Jucevičiūtė, N., Banaitytė, I., Vaitkus, A., Balnytė, R., 2019. Preclinical signs of parkinson's disease: A possible association of parkinson's disease with skin and hair features. Med. Hypotheses 127, 100-104.
Kaidoh, T., Inoué, T., 2000. Intercellular junctions between palisade nerve endings and outer root sheath cells of rat vellus hairs. J. Comp. Neurol. 420.
Kalluri, R., Zeisberg, M., 2006. Fibroblasts in cancer. Nature Reviews Cancer 6, 392-401.
Karim, N., Plott, T.J., Durbin-Johnson, B.P., Rocke, D.M., Salemi, M., Phinney, B.S., Goecker, Z.C., Pieterse, M.J.M., Parker, G.J., Rice, R.H., 2021. Elucidation of familial relationships using hair shaft proteomics. Forensic Sci. Int. Genet. 54, 102564.
Kariya, N., Shimomura, Y., Ito, M., 2005. Size polymorphisms in the human ultrahigh sulfur hair keratin-associated protein 4, kap4, gene family. J. Invest. Dermatol. 124, 1111-1118.
Kato, K., Nakayoshi, T., Kurimoto, E., Oda, A., 2020. Mechanisms of deamidation of asparagine residues and effects of main-chain conformation on activation energy. Int J Mol Sci 21, 7035.
Kauser, S., Westgate, G.E., Green, M.R., Tobin, D.J., 2011. Human hair follicle and epidermal melanocytes exhibit striking differences in their aging profile which involves catalase. J Invest Dermatol 131, 979-982.
Kempson, I.M., Lombi, E., 2011. Hair analysis as a biomonitor for toxicology, disease and health status. Chem. Soc. Rev. 40, 3915-3940.
Keogh, E.V., Walsh, R.J., 1965. Rate of greying of human hair. Nature 207, 877-878.
Kim, B.H., Lee, M.J., Lee, W.-Y., Pyo, J., Shin, M.-S., Hwang, G.S., Shin, D., Kim, C.E., Park, E.-S., Kang, K.S., 2021a. Hair growth stimulation effect of centipeda minima extract: Identification of active compounds and anagen-activating signaling pathways. Biomolecules 11, 976.
Kim, J.E., Lee, J.H., Choi, K.H., Lee, W.-S., Choi, G.S., Kwon, O.S., Kim, M.B., Huh, C.-H., Ihm, C.-W., Kye, Y.C., 2013a. Phototrichogram analysis of normal scalp hair characteristics with aging. Eur. J. Dermatol. 23, 849-856.
Kim, S.N., Lee, S.Y., Choi, M.H., Joo, K.M., Kim, S.H., Koh, J.S., Park, W.S., 2013b. Characteristic features of ageing in korean women's hair and scalp. Br. J. Dermatol. 168, 1215-1223.
Kim, S.Y., Cho, W.K., Kim, H.I., Paek, S.H., Jang, S.J., Jo, Y., Choi, H., Lee, J.H., Moh, S.H., 2021b. Transcriptome profiling of human follicle dermal papilla cells in response to porphyra-334 treatment by rna-seq. Evid Based Complement Alternat Med 2021, 6637513.
Kishabongo, A.S., Katchunga, P., Van Aken, E.H., Speeckaert, M.M., Lagniau, S., Husein, D., Taes, Y.E., Delanghe, J.R., 2014. Glycated nail proteins: A new approach for detecting diabetes in developing countries. Trop. Med. Int. Health 19, 58-64.
Kobayashi, K., Igimi, H., 1996. Glycation index of hair for non-invasive estimation of diabetic control. Biol. Pharm. Bull. 19, 487-490.
Kowalczyk, A., Chikina, M., Clark, N., 2022. Complementary evolution of coding and noncoding sequence underlies mammalian hairlessness. eLife 11, e76911.
Laatsch, C.N., Durbin-Johnson, B.P., Rocke, D.M., Mukwana, S., Newland, A.B., Flagler, M.J., Davis, M.G., Eigenheer, R.A., Phinney, B.S., Rice, R.H., 2014. Human hair shaft proteomic profiling: Individual differences, site specificity and cuticle analysis. Peer J 2, e506.
Lagarrigue, S.G., George, J., Questel, E., Lauze, C., Meyer, N., Lagarde, J.-M., Simon, M., Schmitt, A.-M., Serre, G., Paul, C., 2012. In vivo quantification of epidermis

pigmentation and dermis papilla density with reflectance confocal microscopy: Variations with age and skin phototype. Exp. Dermatol. 21, 281-286.
Lai, H.Y., Setyawati, M.I., Ferhan, A.R., Divakarla, S.K., Chua, H.M., Cho, N.-J., Chrzanowski, W., Ng, K.W., 2021. Self-assembly of solubilized human hair keratins. ACS Biomater Sci. Eng. 7, 83-89.
Langbein, L., Rogers, M.A., Winter, H., Praetzel, S., Schweizer, J., 2001. The catalog of human hair keratins. Ii. Expression of the six type ii members in the hair follicle and the combined catalog of human type i and ii keratins. J. Biol. Chem. 276, 35123-35132.
Lay, K., Kume, T., Fuchs, E., 2016. Foxc1 maintains the hair follicle stem cell niche and governs stem cell quiescence to preserve long-term tissue-regenerating potential. Proc. Natl. Acad. Sci. USA 113, E1506-E1515.
Le Stunff, H., Peterson, C., Liu, H., Milstien, S., Spiegel, S., 2002. Sphingosine-1-phosphate and lipid phosphohydrolases. Biochim. Biophys. Acta 1582, 8-17.
Lee, B.S.L., Chan, J.-Y.L., Monselise, A., McElwee, K., Shapiro, J., 2012. Assessment of hair density and caliber in caucasian and asian female subjects with female pattern hair loss by using the folliscope. J. Am. Acad. Dermatol. 66, 166-167.
Lee, Y., Kim, Y.D., Pi, L.Q., Lee, S.Y., Hong, H., Lee, W.S., 2014. Comparison of hair shaft damage after chemical treatment in asian, white european, and african hair. Int J Dermatol 53, 1103-1110.
Lee, Y.J., Rice, R.H., Lee, Y.M., 2006. Proteome analysis of human hair shaft: From protein identification to posttranslational modification. Mol. Cell. Proteomics 5, 789-800.
Li, B., Wu, G., Yang, X., Li, Z., Albasher, G., Alsultan, N., Memon, A.A., Afridi, H.I., 2023. Correlation of endocrine disrupting chemicals with essential elements in biological samples of children (1–5 years) with different infectious diseases and impact on sustainable outdoor activities. Environ. Res. 229, 115781.
Lindner, H., Helliger, W., 2001. Age-dependent deamidation of asparagine residues in proteins. Exp. Gerontol. 36, 1551-1563.
Liu, L.-P., Li, M.-H., Zheng, Y.-W., 2023. Hair follicles as a critical model for monitoring the circadian clock. International Journal of Molecular Sciences 24, 2407.
López-Otín, C., Blasco, M.A., Partridge, L., Serrano, M., Kroemer, G., 2013. The hallmarks of aging. Cell 153, 1194-1217.
Loussouarn, G., El Rawadi, C., Genain, G., 2005. Diversity of hair growth profiles. Int. J. Dermatol. 44, 6-9.
Lu, Z., Xie, Y., Huang, H., Jiang, K., Zhou, B., Wang, F., Chen, T., 2020. Hair follicle stem cells regulate retinoid metabolism to maintain the self-renewal niche for melanocyte stem cells. Elife 9, e52712.
Lynnerup, N., Kjeldsen, H., Heegaard, S., Jacobsen, C., Heinemeier, J., 2008. Radiocarbon dating of the human eye lens crystallines reveal proteins without carbon turnover throughout life. PLoS One 3, e1529.
Ma, K., Tan, Z., Zhang, C., Fu, X., 2016. Mesenchymal stem cells for sweat gland regeneration after burns: From possibility to reality. Burns 42, 492-499.
Ma, Y., He, C., 2022. Exploration of potential lipid biomarkers for age-induced hair greying by lipidomic analyses of hair shaft roots with follicular tissue attached. J. Cosmet. Dermatol. 11, 6118-6123.
Maes, E., Deb-Choudhury, S., Clerens, S., Davis, S.L., Davis, M.G., Marsh, J.M., Willicut, R.J., Dyer, J.M., 2022. Redox proteomics analysis of hair shaft proteins upon hydrothermal and alkaline insult. Int. J. Cosmetic Sci. 44, 82-90.
Mahé, Y.F., Michelet, J.F., Billoni, N., Jarrousse, F., Buan, B., Commo, S., Saint-Léger, D., Bernard, B.A., 2000. Androgenetic alopecia and microinflammation. Int. J. Dermatol. 39, 576-584.

Malgouries, S., Thibaut, S., Bernard, B.A., 2008. Proteoglycan expression patterns in human hair follicle. Br. J. Dermatol. 158, 234-342.
Marmorstein, R., 2003. Structure of set domain proteins: A new twist on histone methylation. Trends Biochem. Sci. 28, 59-62.
Marsden, A.J., Riley, D.R.J., Birkett, S., Rodriguez-Barucg, Q., Guinn, B.A., Carroll, S., Ingle, L., Sathyapalan, T., Beltran-Alvarez, P., 2022. Love is in the hair: Arginine methylation of human hair proteins as novel cardiovascular biomarkers. Amino Acids 54, 591-600.
Martincorena, I., Roshan, A., Gerstung, M., Ellis, P., Van Loo, P., McLaren, S., Wedge, D.C., Fullam, A., Alexandrov, L.B., Tubio, J.M., 2015. High burden and pervasive positive selection of somatic mutations in normal human skin. Science 348, 880-886.
Mastrangelo, A., Barbas, C., 2017. Chronic diseases and lifestyle biomarkers identification by metabolomics, in: Sussulini, A. (Ed.), Metabolomics: From fundamentals to clinical applications. Springer International Publishing, Cham, pp. 235-263.
Matsumura, H., Mohri, Y., Binh, N.T., Morinaga, H., Fukuda, M., Ito, M., Kurata, S., Hoeijmakers, J., Nishimura, E.K., 2016. Hair follicle aging is driven by transepidermal elimination of stem cells via col17a1 proteolysis. Science 351, aad4395.
Maymone, M., Melissa Laughter, Samara Pollock, Iman Khan, Thaís Marques, Rana Abdat, Lynne J. Goldberg, Neelam A. Vashi, 2021. Hair aging in different races and ethnicities. J Clin Aesthet Dermatol. 1;14(1), 38–44.
McElwee, K.J., Huth, A., Kissling, S., Hoffmann, R., 2004. Macrophage-stimulating protein promotes hair growth ex vivo and induces anagen from telogen stage hair follicles in vivo. J. Invest. Dermatol. 123, 34-40.
Medzihradszky, K.F., Kaasik, K., Chalkley, R.J., 2015. Tissue-specific glycosylation at the glycopeptide level. Mol Cell Proteomics 14, 2103-2110.
Memarian, E., t Hart, L.M., Slieker, R.C., Lemmers, R.F.L., van der Heijden, A.A., Rutters, F., Nijpels, G., Schoep, E., Lieverse, A.G., Sijbrands, E.J.G., Wuhrer, M., van Hoek, M., Dotz, V., 2021. Plasma protein n-glycosylation is associated with cardiovascular disease, nephropathy, and retinopathy in type 2 diabetes. BMJ Open Diabetes Res Care 9.
Mendelsohn, A.R., Larrick, J.W., 2020. The danger of being too sympathetic: Norepinephrine in alzheimer's disease and graying of hair. Rejuvenation Res. 23, 68-72.
Miolo, G., Stocchero, G., Vogliardi, S., Menilli, L., Scrivano, S., Montisci, M., Favretto, D., 2020. A study on photostability of ethyl glucuronide in hair irradiated under artificial sunlight. J. Anal. Toxicol. 44, 58-64.
Mistry, D.A., Haklani, J., French, P.W., 2012. Identification of breast cancer-associated lipids in scalp hair. Breast Cancer: Basic and Clinical Research 6, BCBCR. S9607.
Mizukoshi, K., Kuribayashi, M., Hirayama, K., Yabuzaki, J., Kurosumi, M., Hamanaka, Y., 2021. Examination of age-related changes of viscoelasticity in the dermis and subcutaneous fat layer using ultrasound elastography. Skin Res. Technol. 27, 618-626.
Mohamed Nasir, N., Hiji, J., Jayapalan, J.J., Hashim, O.H., 2020. Potential use of human hair shaft keratin peptide signatures to distinguish gender and ethnicity. Peer J 8, e8248.
Møller, P., Danielsen, P.H., Karottki, D.G., Jantzen, K., Roursgaard, M., Klingberg, H., Jensen, D.M., Christophersen, D.V., Hemmingsen, J.G., Cao, Y., Loft, S., 2014. Oxidative stress and inflammation generated DNA damage by exposure to air pollution particles. Mutat Res Rev Mutat Res 762, 133-166.
Morice-Picard, F., Lasseaux, E., Cailley, D., Gros, A., Toutain, J., Plaisant, C., Simon, D., François, S., Gilbert-Dussardier, B., Kaplan, J., Rooryck, C., Lacombe, D., Arveiler, B., 2014. High-resolution array-cgh in patients with oculocutaneous albinism identifies new deletions of the tyr, oca2, and slc45a2 genes and a complex rearrangement of the oca2 gene. Pigment Cell Melanoma Res 27, 59-71.

Morinaga, H., Mohri, Y., Grachtchouk, M., Asakawa, K., Matsumura, H., Oshima, M., Takayama, N., Kato, T., Nishimori, Y., Sorimachi, Y., Takubo, K., Suganami, T., Iwama, A., Iwakura, Y., Dlugosz, A.A., Nishimura, E.K., 2021. Obesity accelerates hair thinning by stem cell-centric converging mechanisms. Nature 595, 266-271.

Mosley, J.G., Gibbs, A.C., 1996. Premature grey hair and hair loss among smokers: A new opportunity for health education? BMJ 313, 1616.

Na, C.H., Sharma, N., Madugundu, A.K., Chen, R., Aksit, M.A., Rosson, G.D., Cutting, G.R., Pandey, A., 2019. Integrated transcriptomic and proteomic analysis of human eccrine sweat glands identifies missing and novel proteins. Mol Cell Proteomics 18, 1382-1395.

Nanba, D., Sakabe, J.-I., Mosig, J., Brouard, M., Toki, F., Shimokawa, M., Kamiya, M., Braschler, T., Azzabi, F., Droz-Georget Lathion, S., Johnsson, K., Roy, K., Schmid, C.D., Bureau, J.-B., Rochat, A., Barrandon, Y., Low temperature and mtor inhibition favor stem cell maintenance in human keratinocyte cultures. EMBO reports n/a, e55439.

Naudin, G., Bastien, P., Mezzache, S., Trehu, E., Bourokba, N., Appenzeller, B.M.R., Soeur, J., Bornschlögl, T., 2019. Human pollution exposure correlates with accelerated ultrastructural degradation of hair fibers. Proc. Natl. Acad. Sci. USA 116, 18410-18415.

Naue, J., Winkelmann, J., Schmidt, U., Lutz-Bonengel, S., 2021. Analysis of age-dependent DNA methylation changes in plucked hair samples using massive parallel sequencing. Rechtsmedizin 31, 226-233.

Nicu, C., O'Sullivan, J.D.B., Ramos, R., Timperi, L., Lai, T., Farjo, N., Farjo, B., Pople, J., Bhogal, R., Hardman, J.A., Plikus, M.V., Ansell, D.M., Paus, R., 2021. Dermal adipose tissue secretes hgf to promote human hair growth and pigmentation. J Invest Dermatol 141, 1633-1645.e1613.

Nishimura, E.K., Granter, S.R., Fisher, D.E., 2005. Mechanisms of hair graying: Incomplete melanocyte stem cell maintenance in the niche. Science 307, 720-724.

Noppe, G., de Rijke, Y.B., Dorst, K., van den Akker, E.L., van Rossum, E.F., 2015. Lc-ms/ms-based method for long-term steroid profiling in human scalp hair. Clin Endocrinol 83, 162-166.

O'Sullivan, J.D.B., Nicu, C., Picard, M., Chéret, J., Bedogni, B., Tobin, D.J., Paus, R., 2021. The biology of human hair greying. Biol. Rev. Camb. Philos. Soc. 96, 107-128.

Palazzi, P., Mezzache, S., Bourokba, N., Hardy, E.M., Schritz, A., Bastien, P., Emond, C., Li, J., Soeur, J., Appenzeller, B.M., 2018. Exposure to polycyclic aromatic hydrocarbons in women living in the chinese cities of baoding and dalian revealed by hair analysis. Environ. Int. 121, 1341-1354.

Palmer-Bacon, J., Willis-Esqueda, C., Spaulding, W.D., 2019. Stress, trauma, racial/ethnic group membership, and hpa function: Utility of hair cortisol. Am. J. Orthopsychiatry.

Panhard, S., Lozano, I., Loussouarn, G., 2012. Greying of the human hair: A worldwide survey, revisiting the '50' rule of thumb. Br. J. Dermatol. 167, 865-873.

Parekh, R., Roitt, I., Isenberg, D., Dwek, R., Rademacher, T., 1988. Age-related galactosylation of the n-linked oligosaccharides of human serum igg. J Exp Med 167, 1731-1736.

Parker, G.J., Leppert, T., Anex, D.S., Hilmer, J.K., Matsunami, N., Baird, L., Stevens, J., Parsawar, K., Durbin-Johnson, B.P., Rocke, D.M., Nelson, C., Fairbanks, D.J., Wilson, A.S., Rice, R.H., Woodward, S.R., Bothner, B., Hart, B.R., Leppert, M., 2016. Demonstration of protein-based human identification using the hair shaft proteome, PLoS One, pp. e0160653, 0160610.0161371/journal.pone.0160653.

Parry, D.A., Strelkov, S.V., Burkhard, P., Aebi, U., Herrmann, H., 2007. Towards a molecular description of intermediate filament structure and assembly. Exp. Cell Res. 313, 2204-2216.

Paton, B., Suarez, M., Herrero, P., Canela, N., 2021. Glycosylation biomarkers associated with age-related diseases and current methods for glycan analysis. Int J Mol Sci 22.
Paus, R., 2011. A neuroendocrinological perspective on human hair follicle pigmentation. Pigment Cell Melanoma Res 24, 89-106.
Paus, R., Cotsarelis, G., 1999. The biology of hair follicles. New Engl. J. Med. 341, 491-497.
Paus, R., Langan, E.A., Vidali, S., Ramot, Y., Andersen, B., 2014. Neuroendocrinology of the hair follicle: Principles and clinical perspectives. Trends Mol. Med. 20, 559-570.
Pavan, W.J., Sturm, R.A., 2019. The genetics of human skin and hair pigmentation. Annu Rev Genomics Hum Genet 20, 41-72.
Peters, E.M., Liezmann, C., Spatz, K., Ungethüm, U., Kuban, R.-J., Daniltchenko, M., Kruse, J., Imfeld, D., Klapp, B.F., Campiche, R., 2013. Profiling mrna of the graying human hair follicle constitutes a promising state-of-the-art tool to assess its aging: An exemplary report. J. Invest. Dermatol. 133, 1150-1160.
Pflieger, D., Chabane, S., Gaillard, O., Bernard, B.A., Ducoroy, P., Rossier, J., Vinh, J., 2006. Comparative proteomic analysis of extracellular matrix proteins secreted by two types of skin fibroblasts. Proteomics 6, 5868-5879.
Pichini, S., García-Algar, O., Alvarez, A.-T., Mercadal, M., Mortali, C., Gottardi, M., Svaizer, F., Pacifici, R., 2014. Pediatric exposure to drugs of abuse by hair testing: Monitoring 15 years of evolution in spain. Int. J. Env. Res. Public Health 11, 8267-8275.
Piérard-Franchimont, C., Loussouarn, G., Panhard, S., Saint Léger, D., Mellul, M., Piérard, G.E., 2013. Immunohistochemical patterns in the interfollicular caucasian scalps: Influences of age, gender, and alopecia. Biomed. Res. Int. 2013.
Piérard, G.E., Piérard-Franchimont, C., Marks, R., Paye, M., Rogiers, V., 2000. Eemco guidance for the in vivo assessment of skin greasiness. The eemco group. Skin Pharmacol. Appl. Skin Physiol. 13, 372-389.
Plott, T.J., Karim, N., Durbin-Johnson, B.P., Swift, D.P., Scott Youngquist, R., Salemi, M., Phinney, B.S., Rocke, D.M., Davis, M.G., Parker, G.J., Rice, R.H., 2020. Age-related changes in hair shaft protein profiling and genetically variant peptides. Forensic Sci. Int. Genet. 47, 102309.
Poblet, E., Jimenez, F., Escario- Travesedo, E., Hardman, J.A., Hernández- Hernández, I., Agudo- Mena, J.L., Cabrera- Galvan, J.J., Nicu, C., Paus, R., 2018. Eccrine sweat glands associate with the human hair follicle within a defined compartment of dermal white adipose tissue. Br. J. Dermatol. 178, 1163-1172.
Porta, T., Grivet, C., Kraemer, T., Varesio, E., Hopfgartner, G., 2011. Single hair cocaine consumption monitoring by mass spectrometric imaging. Anal. Chem. 83, 4266-4272.
Pötsch, L., Skopp, G., Moeller, M., 1997. Influence of pigmentation on the codeine content of hair fibers in guinea pigs. Journal of Forensic Science 42, 1095-1098.
Praetorius, C., Grill, C., Stacey, S.N., Metcalf, A.M., Gorkin, D.U., Robinson, K.C., Van Otterloo, E., Kim, R.S., Bergsteinsdottir, K., Ogmundsdottir, M.H., 2013. A polymorphism in irf4 affects human pigmentation through a tyrosinase-dependent mitf/tfap2a pathway. Cell 155, 1022-1033.
Pragst, F., Balikova, M.A., 2006a. State of the art in hair analysis for detection of drug and alcohol abuse. Clin. Chim. Acta 370, 17-49.
Pragst, F., Balikova, M.A., 2006b. State of the art in hair analysis for detection of drug and alcohol abuse. Clinica Chimica Acta 370, 17-49.
Pss, R., Madhunapantula, S.V., Betkerur, J.B., Bovilla, V.R., Shastry, V., 2022. Melanogenesis markers expression in premature graying of hair: A cross-sectional study. Skin Pharmacol. Physiol. 35, 180-186.

Quan, T., Qin, Z., Xia, W., Shao, Y., Voorhees, J.J., Fisher, G.J., 2009. Matrix-degrading metalloproteinases in photoaging. Journal of Investigative Dermatology Symposium Proceedings 14, 20-24.

Rabbani, P., Takeo, M., Chou, W., Myung, P., Bosenberg, M., Chin, L., Taketo, M.M., Ito, M., 2011. Coordinated activation of wnt in epithelial and melanocyte stem cells initiates pigmented hair regeneration. Cell 145, 941-955.

Rajput, R., 2015. Understanding hair loss due to air pollution and the approach to management. Hair Ther Transplant 5, 2.

Rebecca, M.P., Declan, P.L., Gayle, H., Jane, R., Neil, J.W., Lane, E.B., Colin, A.B.J., W.H, Neil, V.W., Julia, R., Thomas, M.M., 2002. Defolliculated (dfl): A dominant mouse mutation leading to poor sebaceous gland differentiation and total elimination of pelage follicles. J. Invest. Dermatol. 119, 32-37.

Rishikaysh, P., Dev, K., Diaz, D., Qureshi, W.M., Filip, S., Mokry, J., 2014. Signaling involved in hair follicle morphogenesis and development. Int J Mol Sci 15, 1647-1670.

Rittié, L., Fisher, G.J., 2015. Natural and sun-induced aging of human skin. Cold Spring Harb. Perspect. Med. 5, a015370.

Robbins, C., Mirmirani, P., Messenger, A.G., Birch, M.P., Youngquist, R.S., Tamura, M., Filloon, T., Luo, F., Dawson Jr, T.L., 2012. What women want – quantifying the perception of hair amount: An analysis of hair diameter and density changes with age in caucasian women. Br. J. Dermatol. 167, 324-332.

Robinson, N.E., Robinson, A.B., 2001. Molecular clocks. Proc. Natl. Acad. Sci. USA 98, 944-949.

Rogers, G.E., 2019. Known and unknown features of hair cuticle structure: A brief review. Cosmetics 6, 32.

Rogers, M.A., Langbein, L., Praetzel-Wunder, S., Giehl, K., 2008. Characterization and expression analysis of the hair keratin associated protein kap26.1. British Journal of Dermatology 159, 725-729.

Rosenberg, A.M., Rausser, S., Ren, J., Mosharov, E.V., Sturm, G., Ogden, R.T., Patel, P., Kumar Soni, R., Lacefield, C., Tobin, D.J., Paus, R., Picard, M., 2021. Quantitative mapping of human hair greying and reversal in relation to life stress. eLife 10, e67437.

Rosenquist, T.A., Martin, G.R., 1996. Fibroblast growth factor signalling in the hair growth cycle: Expression of the fibroblast growth factor receptor and ligand genes in the murine hair follicle. Developmental dynamics: an official publication of the American Association of Anatomists 205, 379-386.

Sakata, A., Abe, K., Mizukoshi, K., Gomi, T., Okuda, I., 2018. Relationship between the retinacula cutis and sagging facial skin. Skin Res. Technol. 24, 93-98.

Sandu, C., Dumas, M., Malan, A., Sambakhe, D., Marteau, C., Nizard, C., Schnebert, S., Perrier, E., Challet, E., Pévet, P., 2012. Human skin keratinocytes, melanocytes, and fibroblasts contain distinct circadian clock machineries. Cell. Mol. Life Sci. 69, 3329-3339.

Santaularia, N.J., Kunin-Batson, A., Sherwood, N.E., Gunnar, M.R., French, S.A., Mason, S.M., 2023. Is economic hardship associated with young children's cortisol levels? Dev. Psychobiol. 65.

Sarin, K.Y., Artandi, S.E., 2007. Aging, graying and loss of melanocyte stem cells. Stem Cell Reviews 3, 212-217.

Sato, Y., Endo, T., 2010. Alteration of brain glycoproteins during aging. Geriatr. Gerontol. Int. 10, S32-S40.

Sauvé, B., Koren, G., Walsh, G., Tokmakejian, S., Van Uum, S.H., 2007. Measurement of cortisol in human hair as a biomarker of systemic exposure. Clin Invest Med 30, E183-191.

Schmidt-Ullrich, R., Paus, R., 2005. Molecular principles of hair follicle induction and morphogenesis. Bioessays 27, 247-261.
Schneider, M.R., Schmidt-Ullrich, R., Paus, R., 2009. The hair follicle as a dynamic miniorgan. Curr. Biol. 19, R132-R142.
Schwab, S.R., Pereira, J.P., Matloubian, M., Xu, Y., Huang, Y., Cyster, J.G., 2005. Lymphocyte sequestration through s1p lyase inhibition and disruption of s1p gradients. Science 309, 1735-1739.
Schwanhäusser, B., Busse, D., Li, N., Dittmar, G., Schuchhardt, J., Wolf, J., Chen, W., Selbach, M., 2011. Global quantification of mammalian gene expression control. Nature 473, 337-342.
Scott, K.S., Nakahara, Y., 2003. A study into the rate of incorporation of eight benzodiazepines into rat hair. Forensic Sci. Int. 133, 47-56.
Seiberg, M., 2013. Age- induced hair greying–the multiple effects of oxidative stress. Int. J. Cosmetic Sci. 35, 532-538.
Shapiro, S., Endicott, S., Province, M., Pierce, J., Campbell, E., 1991. Marked longevity of human lung parenchymal elastic fibers deduced from prevalence of d-aspartate and nuclear weapons-related radiocarbon. J. Clin. Invest. 87, 1828-1834.
Sharov, A., Tobin, D.J., Sharova, T.Y., Atoyan, R., Botchkarev, V.A., 2005. Changes in different melanocyte populations during hair follicle involution (catagen). J. Invest. Dermatol. 125, 1259-1267.
Shi, Y., Luo, L.-F., Liu, X.-M., Zhou, Q., Xu, S.-Z., Lei, T.-C., 2014. Premature graying as a consequence of compromised antioxidant activity in hair bulb melanocytes and their precursors. PLoS One 9, e93589.
Shin, H., Ryu, H.H., Yoon, J., Jo, S., Jang, S., Choi, M., Kwon, O., Jo, S.J., 2015. Association of premature hair graying with family history, smoking, and obesity: A cross-sectional study. J. Am. Acad. Dermatol. 72, 321-327.
Shin, J.W., Kwon, S.H., Choi, J.Y., Na, J.I., Huh, C.H., Choi, H.R., Park, K.C., 2019. Molecular mechanisms of dermal aging and antiaging approaches. Int J Mol Sci 20.
Shomode, A., Yagi, M., Naito, J., Moniruzzaman M, T, k., Y, Y., 2014. Hair protein glycation reduces hair breaking strength. Glycative Stress Res. 1, 037-045.
Siegl-Cachedenier , I., Flores , I., Klatt , P., Blasco , M.A., 2007. Telomerase reverses epidermal hair follicle stem cell defects and loss of long-term survival associated with critically short telomeres. J. Cell Biol. 179, 277-290.
Sikkink, S.K., Mine, S., Freis, O., Danoux, L., Tobin, D.J., 2020. Stress-sensing in the human greying hair follicle: Ataxia telangiectasia mutated (atm) depletion in hair bulb melanocytes in canities-prone scalp. Sci Rep 10, 18711.
Sinclair, R., Flagler, M., Jones, L., Rufaut, N., Davis, M., 2012. The proteomic profile of hair damage. Br. J. Dermatol. 166, 27-32.
Slominski, A., Tobin, D.J., Shibahara, S., Wortsman, J., 2004. Melanin pigmentation in mammalian skin and its hormonal regulation. Physiol. Rev. 84, 1155-1228.
Slominski, A., Wortsman, J., Plonka, P.M., Schallreuter, K.U., Paus, R., Tobin, D.J., 2005. Hair follicle pigmentation. The Journal of investigative dermatology 124, 13-21.
Slominski, A.T., Hardeland, R., Zmijewski, M.A., Slominski, R.M., Reiter, R.J., Paus, R., 2018. Melatonin: A cutaneous perspective on its production, metabolism, and functions. J. Invest. Dermatol. 138, 490-499.
Spalding, K.L., Buchholz, B.A., Bergman, L.-E., Druid, H., Frisén, J., 2005. Age written in teeth by nuclear tests. Nature 437, 333-334.
Steingrímsson, E., Copeland, N.G., Jenkins, N.A., 2005. Melanocyte stem cell maintenance and hair graying. Cell 121, 9-12.
Stenn, K.S., Paus, R., 2001. Controls of hair follicle cycling. Physiol. Rev. 81, 449-494.

Stone, R.C., Aviv, A., Paus, R., 2021. Telomere dynamics and telomerase in the biology of hair follicles and their stem cells as a model for aging research. J. Invest. Dermatol. 141, 1031-1040.
Subbaiah, R.S., Kerk, S.K., Lian, Y., Lunny, D., Sze, S.K., Ng, K.W., Schmidtchen, A., Adav, S.S., 2018. Identification of antibacterial components in human hair shafts. Acta Derm. Venereol. 98, 708-710.
Sulek, K., Han, T.-L., Villas-Boas, S.G., Wishart, D.S., Soh, S.-E., Kwek, K., Gluckman, P.D., Chong, Y.-S., Kenny, L.C., Baker, P.N., 2014. Hair metabolomics: Identification of fetal compromise provides proof of concept for biomarker discovery. Theranostics 4, 953-959.
Swayambhu, M., Kümmerli, R., Arora, N., 2023. Microbiome-based stain analyses in crime scenes. Applied and Environmental Microbiology, e01325-01322.
Takahashi-Iwanaga, H., 2000. Three-dimensional microanatomy of longitudinal lanceolate endings in rat vibrissae. J Comp Neurol 426, 259-269.
Takahashi, R., Grzenda, A., Allison, T.F., Rawnsley, J., Balin, S.J., Sabri, S., Plath, K., Lowry, W.E., 2020. Defining transcriptional signatures of human hair follicle cell states. J Invest Dermatol 140, 764-773.e764.
Tang, L., Li, J., Lin, X., Wu, W., Kang, K., Fu, W., 2012. Oxidation levels differentially impact melanocytes: Low versus high concentration of hydrogen peroxide promotes melanin synthesis and melanosome transfer. Dermatology 224, 145-153.
Tejera, A.M., d'Alcontres, M.S., Thanasoula, M., Marion, R.M., Martinez, P., Liao, C., Flores, J.M., Tarsounas, M., Blasco, M.A., 2010. Tpp1 is required for tert recruitment, telomere elongation during nuclear reprogramming, and normal skin development in mice. Developmental cell 18, 775-789.
Thibaut, S., De Becker, E., Bernard, B.A., Huart, M., Fiat, F., Baghdadli, N., Luengo, G.S., Leroy, F., Angevin, P., Kermoal, A.M., Muller, S., Peron, M., Provot, G., Kravtchenko, S., Saint-Léger, D., Desbois, G., Gauchet, L., Nowbuth, K., Galliano, A., Kempf, J.Y., Silberzan, I., 2010. Chronological ageing of human hair keratin fibres. Int. J. Cosmetic Sci. 32, 422-434.
Tobin, D., Paus, R., 2001. Graying: Gerontobiology of the hair follicle pigmentary unit. Exp. Gerontol. 36, 29-54.
Tobin, D.J., 2005. Hair in toxicology: An important bio-monitor. Royal Society of Chemistry.
Tobin, D.J., 2009. Aging of the hair follicle pigmentation system. Int. J. Trichology 1, 83-93.
Tobin, D.J., 2015. Age-related hair pigment loss. Alopecias-Practical Evaluation and Management 47, 128-138.
Tobin, D.J., Kauser, S., 2005. Hair melanocytes as neuro-endocrine sensors--pigments for our imagination. Mol. Cell. Endocrinol. 243, 1-11.
Tokunaga, S., Tanamachi, H., Ishikawa, K., 2019. Degradation of hair surface: Importance of 18-mea and epicuticle. Cosmetics. , p. 31.
Trüeb, R.M., 2003. Association between smoking and hair loss: Another opportunity for health education against smoking? Dermatology 206, 189-191.
Trüeb, R.M., 2015. The impact of oxidative stress on hair. Int. J. Cosmetic Sci. 37, 25-30.
Trüeb, R.M., 2021. Oxidative stress and its impact on skin, scalp and hair. Int. J. Cosmetic Sci. 43, S9-S13.
Trüeb, R.M., Henry, J.P., Davis, M.G., Schwartz, J.R., 2018a. Scalp condition impacts hair growth and retention via oxidative stress. Int. J. Trichology 10, 262-270.
Trüeb, R.M., Rezende, H.D., Dias, M.F.R.G., 2018b. A comment on the science of hair aging. Int. J. Trichology 10, 245.
Van Neste, D., Tobin, D.J., 2004. Hair cycle and hair pigmentation: Dynamic interactions and changes associated with aging. Micron 35, 193-200.

Vanhooren, V., Dewaele, S., Libert, C., Engelborghs, S., De Deyn, P.P., Toussaint, O., Debacq-Chainiaux, F., Poulain, M., Glupczynski, Y., Franceschi, C., 2010. Serum n-glycan profile shift during human ageing. Exp. Gerontol. 45, 738-743.
Vasconcelos, A., Cavaco-Paulo, A., 2013. The use of keratin in biomedical applications. Curr. Drug Targets 14, 612-619.
Waki, M.L., Onoue, K., Takahashi, T., Goto, K., Saito, Y., Inami, K., Makita, I., Angata, Y., Suzuki, T., Yamashita, M., Sato, N., Nakamura, S., Yuki, D., Sugiura, Y., Zaima, N., Goto-Inoue, N., Hayasaka, T., Shimomura, Y., Setou, M., 2011. Investigation by imaging mass spectrometry of biomarker candidates for aging in the hair cortex. PLoS One 6, e26721-e26721.
Wang, H., Wang, J., He, C., 2020. Exploration of potential lipid biomarkers for premature canities by uplc-qtof-ms analyses of hair follicle roots. Exp Dermatol 29, 776-781.
Wang, S., Kang, Y., Qi, F., Jin, H., 2023. Genetics of hair graying with age. Ageing Research Reviews 89, 101977.
Wang, S., Wang, Z., Foo, S.E.M., Tan, N.S., Yuan, Y., Lin, W., Zhang, Z., Ng, K.W., 2015. Culturing fibroblasts in 3d human hair keratin hydrogels. ACS Applied Materials & Interfaces 7, 5187-5198.
Wei, Z., Batagov, A.O., Schinelli, S., Wang, J., Wang, Y., El Fatimy, R., Rabinovsky, R., Balaj, L., Chen, C.C., Hochberg, F., 2017. Coding and noncoding landscape of extracellular rna released by human glioma stem cells. Nat. Commun. 8, 1145.
Williams, R., Westgate, G.E., Pawlus, A.D., Sikkink, S.K., Thornton, M.J., 2021. Age-related changes in female scalp dermal sheath and dermal fibroblasts: How the hair follicle environment impacts hair aging. J. Invest. Dermatol. 141, 1041-1051.
Wishart, D.S., Feunang, Y.D., Marcu, A., Guo, A.C., Liang, K., Vázquez-Fresno, R., Sajed, T., Johnson, D., Li, C., Karu, N., 2018. Hmdb 4.0: The human metabolome database for 2018. Nucleic Acids Res. 46, D608-D617.
Won, C.H., Kwon, O.S., Kang, Y.J., Yoo, H.G., Lee, D.H., Chung, J.H., Kim, K.H., Park, W.S., Park, N.H., Cho, K., Kwon, S.O., Choi, J.S., Eun, H.C., 2012. Comparative secretome analysis of human follicular dermal papilla cells and fibroblasts using shotgun proteomics. BMB Rep 45, 253-258.
Wood, J.M., Decker, H., Hartmann, H., Chavan, B., Rokos, H., Spencer, J.D., Hasse, S., Thornton, M.J., Shalbaf, M., Paus, R., Schallreuter, K.U., 2009. Senile hair graying: H2o2-mediated oxidative stress affects human hair color by blunting methionine sulfoxide repair. FASEB J. 23, 2065-2075.
Wright, K.D., Hickman, R., Laudenslager, M.L., 2015. Hair cortisol analysis: A promising biomarker of hpa activation in older adults. The Gerontologist 55, S140-S145.
Wu, S., Yu, Y., Liu, C., Zhang, X., Zhu, P., Peng, Y., Yan, X., Li, Y., Hua, P., Li, Q., Wang, S., Zhang, L., 2022. Single-cell transcriptomics reveals lineage trajectory of human scalp hair follicle and informs mechanisms of hair graying. Cell Discovery 8, 49.
Xie, L., Jin, L., Feng, J., Lv, J., 2017. The expression of aqp5 and uts in the sweat glands of uremic patients. Biomed. Res. Int. 2017.
Yamamoto, A., Serizawa, S., Ito, M., Sato, Y., 1987. Effect of aging on sebaceous gland activity and on the fatty acid composition of wax esters. J Invest Dermatol 89, 507-512.
Yamanishi, H., Iwabuchi, T., 2023. Three-dimensional correlative light and focused ion beam scanning electron microscopy reveals the distribution and ultrastructure of lanceolate nerve endings surrounding terminal hair follicles in human scalp skin. Journal of Anatomy 242, 1012-1028.
Yang, C.-C., Cotsarelis, G., 2010. Review of hair follicle dermal cells. J. Dermatol. Sci. 57, 2-11.

Yang, J.H., Hayano, M., Griffin, P.T., Amorim, J.A., Bonkowski, M.S., Apostolides, J.K., Salfati, E.L., Blanchette, M., Munding, E.M., Bhakta, M., Chew, Y.C., Guo, W., Yang, X., Maybury-Lewis, S., Tian, X., Ross, J.M., Coppotelli, G., Meer, M.V., Rogers-Hammond, R., Vera, D.L., Lu, Y.R., Pippin, J.W., Creswell, M.L., Dou, Z., Xu, C., Mitchell, S.J., Das, A., O'Connell, B.L., Thakur, S., Kane, A.E., Su, Q., Mohri, Y., Nishimura, E.K., Schaevitz, L., Garg, N., Balta, A.M., Rego, M.A., Gregory-Ksander, M., Jakobs, T.C., Zhong, L., Wakimoto, H., El Andari, J., Grimm, D., Mostoslavsky, R., Wagers, A.J., Tsubota, K., Bonasera, S.J., Palmeira, C.M., Seidman, J.G., Seidman, C.E., Wolf, N.S., Kreiling, J.A., Sedivy, J.M., Murphy, G.F., Green, R.E., Garcia, B.A., Berger, S.L., Oberdoerffer, P., Shankland, S.J., Gladyshev, V.N., Ksander, B.R., Pfenning, A.R., Rajman, L.A., Sinclair, D.A., 2023. Loss of epigenetic information as a cause of mammalian aging. Cell 186, 305-326.e327.

Yang, Y., Bedford, M.T., 2013. Protein arginine methyltransferases and cancer. Nature Reviews Cancer 13, 37-50.

Yang, Y., Li, X., Zhang, T., Xu, D., 2022. Rip kinases and necroptosis in aging and aging-related diseases. Life Medicine 1, 2-20.

Yano, K., Brown, L.F., Lawler, J., Miyakawa, T., Detmar, M., 2003. Thrombospondin-1 plays a critical role in the induction of hair follicle involution and vascular regression during the catagen phase. J Invest Dermatol 120, 14-19.

Zhang, B., Ma, S., Rachmin, I., He, M., Baral, P., Choi, S., Gonçalves, W.A., Shwartz, Y., Fast, E.M., Su, Y., Zon, L.I., Regev, A., Buenrostro, J.D., Cunha, T.M., Chiu, I.M., Fisher, D.E., Hsu, Y.-C., 2020. Hyperactivation of sympathetic nerves drives depletion of melanocyte stem cells. Nature 577, 676-681.

Zhang, H., Zhu, N.-X., Huang, K., Cai, B.-Z., Zeng, Y., Xu, Y.-M., Liu, Y., Yuan, Y.-P., Lin, C.-M., 2016. Itraq-based quantitative proteomic comparison of early- and late-passage human dermal papilla cell secretome in relation to inducing hair follicle regeneration. PLoS One 11, e0167474-e0167474.

Zhang, J., Liu, W., Simayijiang, H., Hu, P., Yan, J., 2022a. Application of microbiome in forensics. Genomics, Proteomics & Bioinformatics.

Zhang, J., Xin, L., Shan, B., Chen, W., Xie, M., Yuen, D., Zhang, W., Zhang, Z., Lajoie, G.A., Ma, B., 2012. Peaks db: De novo sequencing assisted database search for sensitive and accurate peptide identification. Mol. Cell. Proteomics 11, M111. 010587.

Zhang, P., Kling, R.E., Ravuri, S.K., Kokai, L.E., Rubin, J.P., Chai, J.K., Marra, K.G., 2014. A review of adipocyte lineage cells and dermal papilla cells in hair follicle regeneration. J Tissue Eng 5, 2041731414556850.

Zhang, Y., Li, Y., Xia, Q., Li, Y., Jin, S., Mao, Q., Liu, C., Fan, X., Lin, H., 2022b. Cell membrane-coated human hair nanoparticles for precise disease therapies. Journal of Nanobiotechnology 20, 480.

Zheng, Q., Zhang, X., Bao, P., Zhou, X., Chu, M., Guo, X., Liang, C., Pan, H., Yan, P., 2022. Understanding mammalian hair follicle ecosystems by single-cell rna sequencing. Animals 12, 2409.

Zouboulis, C.C., Fimmel, S., Ortmann, J., Turnbull, J.R., Boschnakow, A., 2003. Sebaceous glands. Neonatal Skin: Structure and Function. 2nd ed. Basel: Taylorand Francis, 59-88.

Zwick, R.K., Guerrero-Juarez, C.F., Horsley, V., Plikus, M.V., 2018. Anatomical, physiological, and functional diversity of adipose tissue. Cell Metab. 27, 68-83.

Figure legends

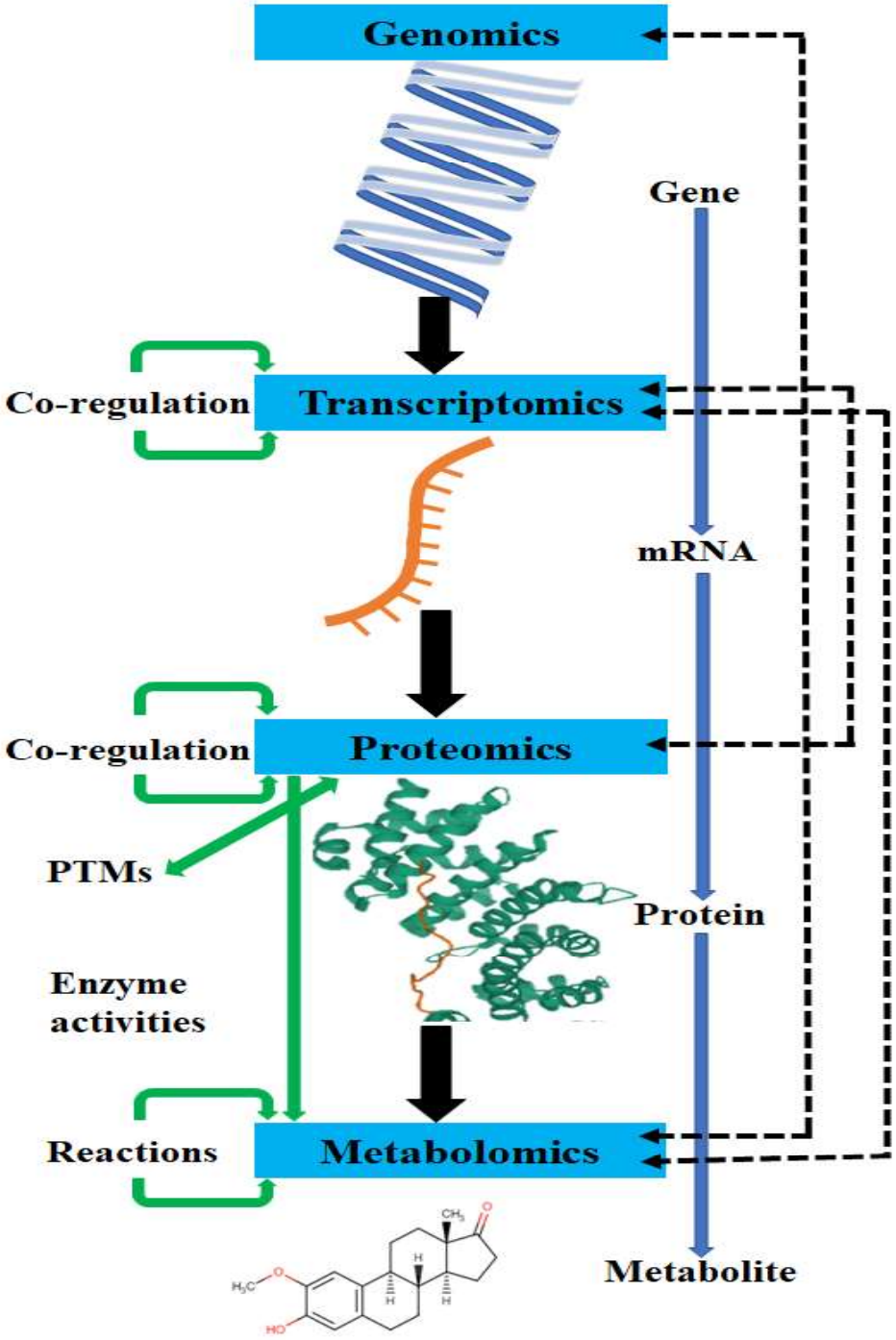
Figure 1. The "Omics" approach. The technique is shown by a solid black arrow, information flow by a blue arrow, regulation\dependencies by a green arrow, and observed association by a dashed line arrow. Molecular information (gene) is transcribed to mRNA, which is translated to proteins processed along with other biomolecules to produce a milieu of metabolites.

Figure 2. Schematics of the anatomy and physiology of the hair follicle (HF) and hair shaft (HS).

Figure 3. Biosynthetic pathways of eumelanin and pheomelanin production. (Adopted from (Ito and Wakamatsu, 2011).

Figure 4. The balance and imbalance of oxidative stress during melanogenesis. The melanocytes in a pigmented young hair follicle cope with oxidative stress, while an imbalance of oxidative stress results in an old follicle due to cumulative effects of intrinsic and extrinsic insults

Figure 5. A typical integrated immunohistomorphometric and omics workflow. Using samples like HF, HFCS or other HF glands, structural biology and omics data integration could provide a unique in-depth structure-function relationship, which could be useful in understanding the aging process, disease pathology, and may provide therapeutic targets.



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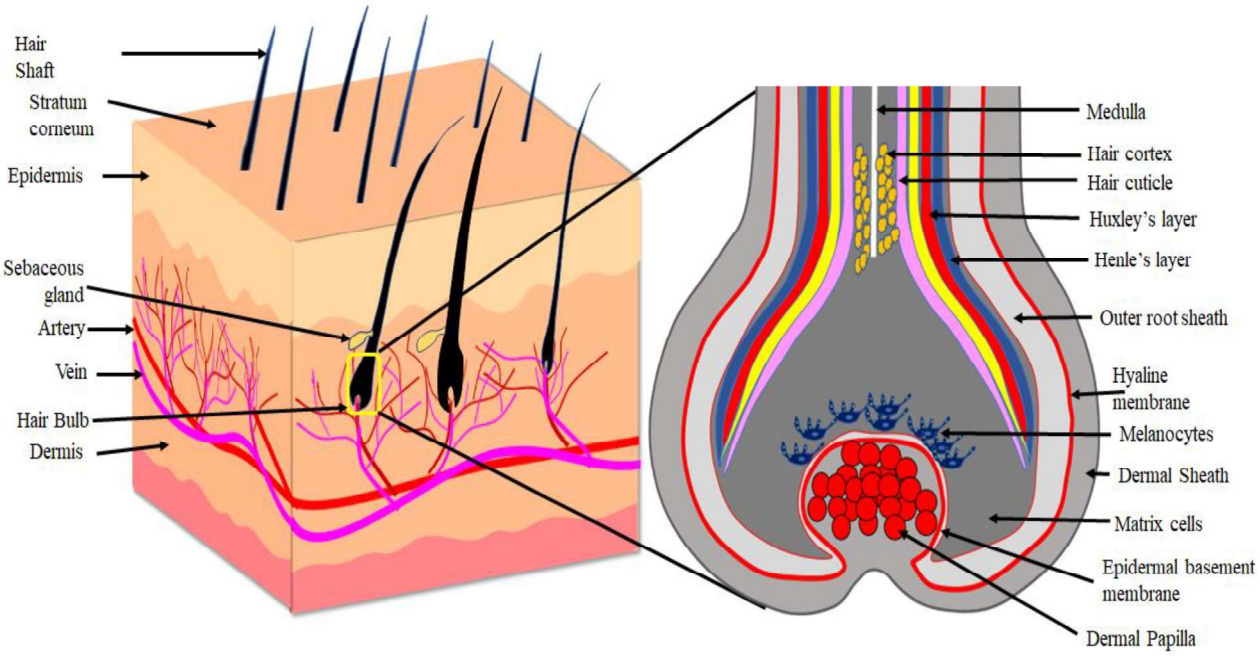

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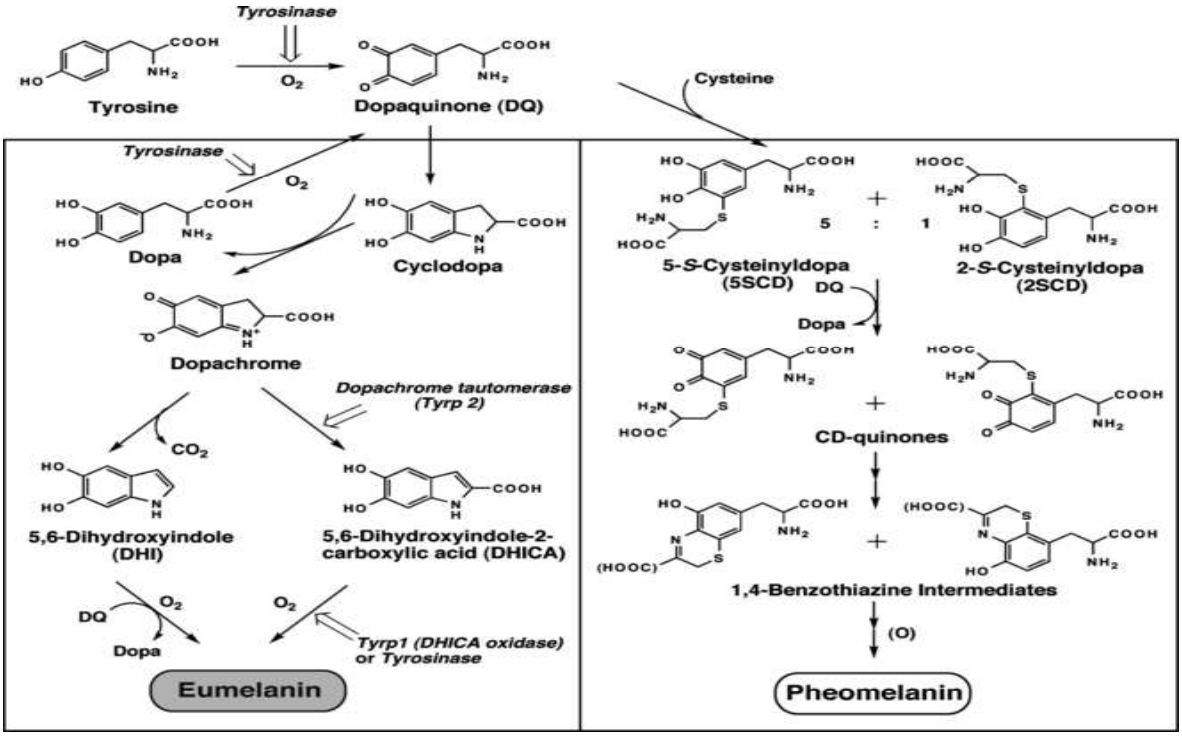

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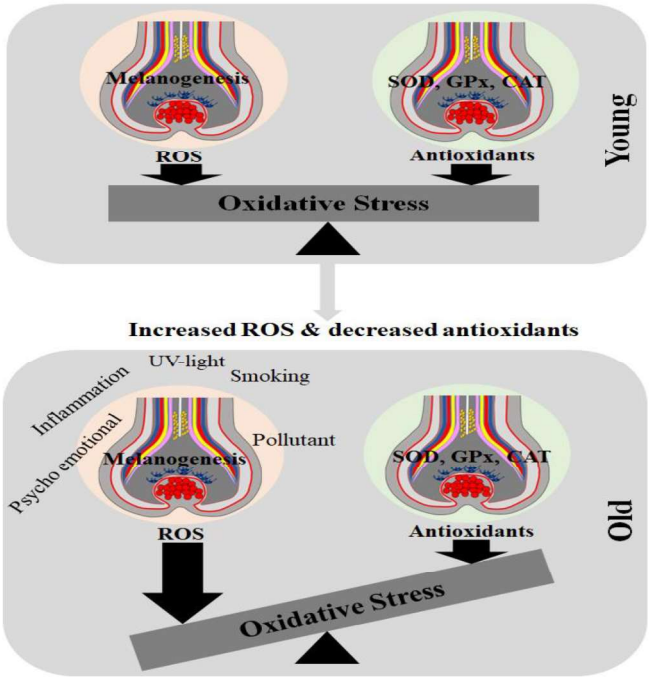

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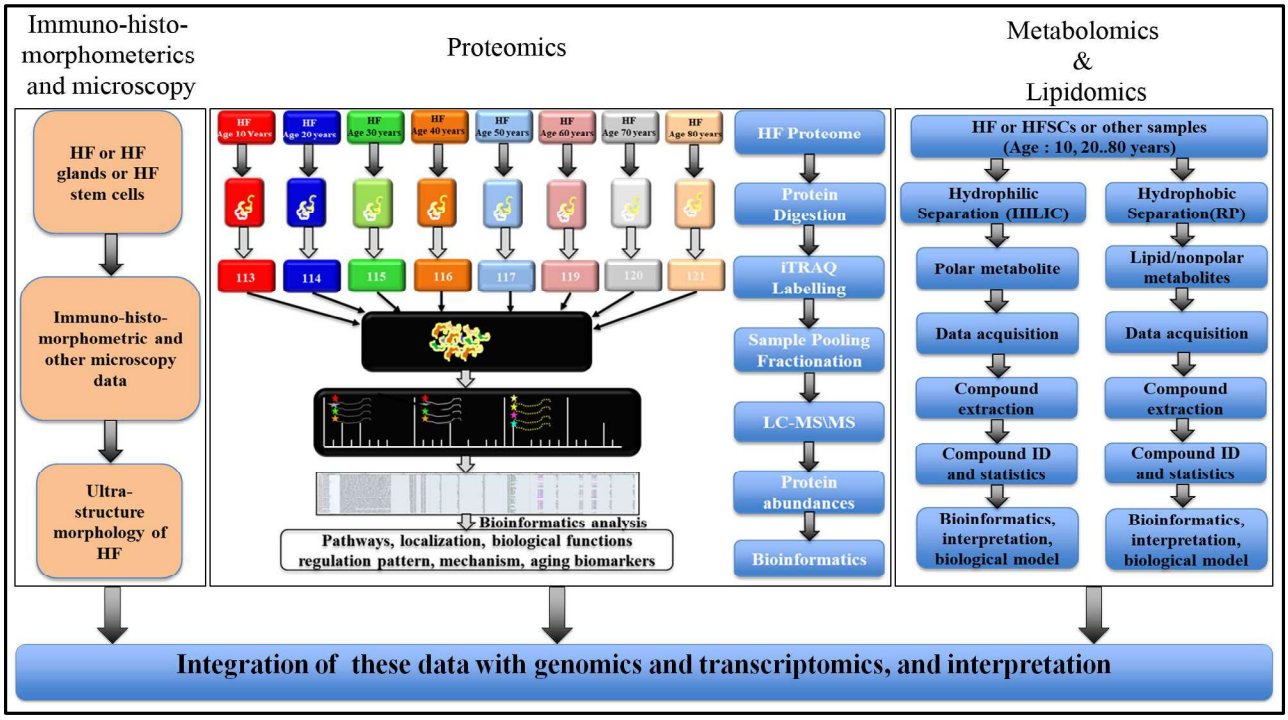

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Table1. Use of hair as a specimen in biomarker discovery, clinical, forensics and aging studies.

No	Pathological Condition\Application	Study objective	Analytical Platform	Altered protein\metabolites	Conclusive Remark	Reference
1	Endocrine disruption in children	Correlation between essential trace elements and toxic metals	Atomic absorption spectrophotometry	Diseased children had lower essential trace elements (iron, copper, and zinc) in their scalp hair.	Exposure to endocrine disrupting chemicals is associated with unfavorable health outcomes	(Li et al., 2023)
2	Baldness and hairlessness	Convergent evolution (Genome-wide scan to study evolutionary genetic basis of reduced hair quantity)	Bioinformatic tools	Upragulated : KRT35 showed no evolutionary rate shifts, FGF11 evolved faster in hairless species. The function of genes related to the dermal papilla preserved	Noncoding regions and microRNAs putatively associated with hair growth	(Kowalczyk et al., 2022)

3	Human age	Predict human age via Hair DNA methylation	Omics	CpGsites from the genes LAG3, SCGN, ELOVL2, KLF14, C1orf132, SLC12A5, GRIA2, and PDE4C associated with age	Chronological age via hair DNA methylation status	(Hao et al., 2021)
4	Cancer, MRSA infection and thrombosis	Hair nanoparticle for therapeutic application	NP delivery system and immunology	Hair nanoparticle (HNP) camouflaged by RBCM or RAWM kills HCC cells and exerts anti-bacterial activity.	HNP ideal material for tumor therapy	(Zhang et al., 2022b)
5	Stress	Stress associated with racial/ethnic discrimination and economic hardship		Analysis of hair specimens in large-scale studies of health, well-being, and racial/ethnic discrimination correlates with cortisol levels or is suggestive.	Hair-based cortisol is useful in health disparities	(Palmer-Bacon et al., 2019) (Santaularia et al., 2023)
6	Cardiovascular disease	Biomarker discovery	LC-MS/MS and ELISA	Levels of protein ArgMe in hair correlate with raised serum concentrations of a well-established cardiovascular biomarker, asymmetric dimethylarginine (ADMA)	ArgMe is a cardiovascular biomarker useful in the prevention	(Marsden et al., 2022)

					and diagnosis of CVD	
7	Hair Cosmetic	Hair damage (heat and alkaline treatments)	LC-MS/MS	Increased redox-related protein modifications upon exposure to higher levels of hydrothermal and alkaline insult	Hair protein damage by environmental or hair treatment-related insults	(Maes et al., 2022)
8	Forensic Science	Gender and ethnicity biomarker	LC-MS/MS	High abundance of KRT81, KRT85 and KRT86 in women	Human hair shaft protein distinguish gender and ethnicity	(Mohamed Nasir et al., 2020)
9	Forensic Science	Individual identification	LC-MS/MS	GVP dependent on the single nucleotide polymorphism profile of the donor genome and can distinguish individuals.	Hair shaft protein profiling and analysis of GVPs distinguish among individuals	(Plott et al., 2020)

10	Intrahepatic Cholestasis of Pregnancy	Biomarker discovery	GS-MS	105 metabolites detected in hair, none were significantly associated with Intrahepatic Cholestasis of Pregnancy	No correlation was found between	(de Seymour et al., 2018)

Table 2 Recent advances and developments in the understanding of hair shaft and hair follicle aging.

Hallmark	Phenotype	Mechanism
Hair Graying	Melanin biosynthesis impacted, diminished production and deposition of pigment in the hair shaft	Analysis of dark and white hair indicated proteins belonging to pigment biosynthesis were impacted. Proteins involved in carbon metabolism, and amino acid biosynthesis were enriched in white hair. Time-dependent progressive functional decline in pigmentary machinery, melanocyte migration defects, failure to self-maintain and proliferate.
Aging biomarker: Post-translational protein modification of HS	Methylated Hf proteins were identified in HF by mass spectrometry	Hair shaft protein methylation was identified but needs more effort to investigate its role in hair thinning, graying and aging
	Deamidated HF proteins identified using mass spectrometry	Protein deamidation is the hallmark of aging and was detected in HF. Deamidated HF proteins were identified but it needs to correlate the extent of deamidation with age. Furthermore, other roles of site-specific deamidation need to be investigated.

	Glycated hair shaft proteins were identified	Glycated hair shaft proteins can be used as biomarkers in monitoring diabetes and long-term glucose control
Hair damage	Biomarkers related to hair damage due to hair treatments and environmental pollutants were identified	Lipids such as C22:1FA, C22FA, C24FA, C24:1FA, C26FA and 18-MEA, were reduced and were in proportion to the severity of hair damage
Hair aging biomarker	Aging biomarker discovery via metabolomics	Dihydrouracil, 3,4-dihydroxymandelic acid and O-phosphoethanolamine were projected as a hair aging biomarkers as their abundances increased with age.
Senescence: hallmark of HF aging	Cellular senescence of dermal papillae cells (DPC), ECM composition of DPC	Dermal papillae cells (DPC) and their ECM protein composition indicate the role of TGF-β signaling pathway in HF regeneration.
Extracellular Matrix: Importance	The hallmark of hair aging is dysfunction of the ECM surrounding the dermal fibroblasts in the scalp.	Dysregulation of ECM proteins of and DCPs and DF impacts the localization of HF bulb.
Senescence: hallmark hair graying	Melanocytes express clock proteins. The accumulation of oxidative stress due to intrinsic and extrinsic factors like UVR	Melanocyte clock proteins regulate anagen-to-catagen transition. The protein expression indicates the molecular clock as an integral part of the hair cycle clock and genes PER1 and or

		BMAL1 regulated the expression of TYRP1 and TYRP2 controlling pigmentation. The accumulation of oxidative stress results in oxidative damage leading to cellular senescence
Inflammation or Microinflmation	Inflammation or microinflammation remains common in hair loss and aging	The factors like environmental pollution, UVR, and other toxins trigger inflammation
Oxidative stress	Hair aging is characterized by altered antioxidant enzymes, and oxidative damage due to exposure of HF and scalp to free radicals, ROS, RON and other environmental factors. Oxidative stress is also generated due to mitochondrial dysfunction. The pigmentary unit is highly susceptible to oxidative stress and ages faster.	Oxidative stress results in ROS production which causes an imbalance in antioxidant defense and initiate cellular senescence. H_2O_2 generated during melanin synthesis and UV irradiation induces oxidative damage and causes melanocyte apoptosis and hair graying.
Stem cells: EpSCs and McSCs and HFSCs	HF bulge maintains epithelial stem cells (EpSCs) and melanocyte stem cells (McSCs) that interact with each other	EpSCs and McSCs communicate population acts in concert to regenerate pigmented hair with each cycle. Wnt signaling plays a key role in these stem cells proliferation and hair regeneration.
	The DNA damage due to oxidative stress or metabolic byproducts of ROS, and inflammation impacts the activity of COL17A1	DNA damage causes proteolysis of COL17A1 in aging HFSCs and leads to its transdermal elimination. The reduction in the level of COL17A1or its inactivation due to proteolysis

		compromises hair follicle stem cell function and its ability to meet the demands of growth and differentiation
HFSC Dysfunction	HFSC dysfunction is associated with hair loss	Aged HFSCs exhibit enhanced resting and elongated growth phases which delays their response to tissue regeneration. This impact hair follicle and initiate a vicious cycle of hair loss.
Scalp microbiome	The oily-natured scalp nurtures unique microflora, the scalp commensal Malassezia that play a key role in hair health and aging.	An imbalance of Malassezia population on healthy scalp causes dandruff and seborrheic dermatitis. Overpopulation of commensal microflora is associated with senescence and inflammation.

Table 3. Biomarkers of hair aging. A summary of the abundances of biomarkers in black and gray hair of young and old individuals, identified using different omics techniques, is listed.

Omics technologies	Method	Biomarkers	Black Hair	Gray Hair	Young (≤30 years)	Old (≥50 years)	References
Genomics	DNA methylation in plucked hair samples (Human plucked head hair)	ELOVL fatty acid elongase 2 (ELOVL2), KLF transcription factor 14 (KLF14), Replication protein A2 (RPA2), Tripartite motif-containing 59 (TRIM59), zyg-11 family member A, cell cycle regulator (ZYG11A)				Higher	(Naue et al., 2021)
Transcriptomics (Gene expression)	Transcriptomics of dermal fibroblast and dermal sheath cells (Hair follicle environment) (Human scalp skin)	Podoplanin (PDPN), Matrix metalloproteinase 1 mRNA, MMP1, Hyaluronic acid synthase 2 (HAS2), Cartilage oligomeric protein (COMP)				Higher	(Williams et al., 2021)
	Transcriptomics of dermal fibroblast and	Alpha-smooth muscle actin (αSMA)			Higher		(Williams et al., 2021)

	dermal sheath cells (Hair follicle environment) (Human scalp skin)						
	Gene expression in the hair follicle (Human hair)	Tyrosinase (TYR), Tyrosinase-related protein-1 (TYRP1), Premelanosome protein (GP100)		Higher			(Pss et al., 2022)
Proteomics	LC-MS\MS of HS (Human hair)	Keratin 40 (KRT40), Keratin 82 (KRT82), Keratin Associated Protein16-1(KRTAP16-1), Keratin Associated Protein 24-1(KRTAP24-1), Keratin Associated Protein3-2 (KRTAP3-2)			Higher		(Plott et al., 2020)
	LC-MS of dermal fibroblast and dermal sheath cells (Hair follicle environment) (Human scalp skin)	Cartilage oligomeric matrix protein, Inactive serine protease PAMR1, Beta-1-glycoprotein, Junction plakoglobin, Afamin, Biglycan, Vitrin, Calmodulin-1,				Higher	(Williams et al., 2021)

		SPARC, Collagen alpha-1(V) chain, Complement factor D, Calumenin, Fibulin-5					
	LC-MS of dermal fibroblast and dermal sheath cells (Hair follicle environment) (Human scalp skin)	Insulin-like growth factor-binding protein 4, Angiopoietin-related protein2, Dihyropyrimidinase-related protein2, Elastin Microfibril Interface-Located Protein 2 (EMILIN-2), Golgi membrane protein 2, Prostaglandin-H2 D-isomarase, Keratin, type II cuticular Hb3			Higher		(Williams et al., 2021)
Metabolomics	UPLC-QTOF-MS of HS (Human hair)	Linoleamide, Cer(d18:0/17:0), 6-Hydroxydelphinidin 3-glucoside, 3-O-alpha-L-rhamnopyranosyl-3-hydroxynonanoyl-3-hydroxydecanoic acid, DG (22:0/22:0/0:0), FAHFA (3:0/2-O-24:0), DG 20:5(5Z,8Z,11Z,14Z,17Z)/20:5(5Z,8Z,11Z,14Z,17Z)/0:0),(9Z)-myristoleoyl-CoA		Higher			(Ma and He, 2022)

		PA(12:0/16:1(9Z)), PS(13:0/20:3(8Z,11Z,14Z)), GlcCer(d18:1/24:1(15Z)), PS(O-16:0/13:0), PS(O-20:0/17:2(9Z,12Z)), Theophylline	Higher				(Ma and He, 2022)
	LC-HRMS of HS (Genuine hair)	Choline (1,2-Aminoalcohols) N-acetylneuramic acid Octanoylcarnitine (C8) Cysteic acid Hexanoylcarnitine (C6) Decanoylcarnitine (C10) Theophylline	Higher				(Eisenbeiss et al., 2020b)
	Hair mass spectrometry imaging (Human hair)	Dihydrouracil (m/z 153.00 tentatively assigned to Dihydrouracil) 3,4-dihydroxymandelic acid (DHMA) (m/z 207.04, identified to be DHMA)			Higher		(Waki et al., 2011)
		O-phosphoethanolamine (m/z 164.00, presumed to be O-phosphoethanolamine)				Higher	(Waki et al., 2011)