Opinion

Protection against chemotherapy-induced alopecia: targeting ATP-binding cassette transporters in the hair follicle?

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Currently, efficacious treatments for chemotherapy-induced alopecia (hair loss) are lacking, and incidences of permanent hair loss following high-dose chemotherapy are on the increase. In this article, we describe mechanisms by which the pharmacological defense status of the hair follicle might be enhanced, thereby reducing the accumulation of cytotoxic cancer drugs and preventing or reducing hair loss and damage. We believe this could be achieved via the selective increase in ATP-binding cassette (ABC) transporter expression within the hair follicle epithelium, following application of topical agonists for regulatory nuclear receptors. Clinical application would require the development of hair follicle-targeted formulations, potentially utilizing nanoparticle technology. This novel approach has the potential to yield entirely new therapeutic options for the treatment and management of chemotherapy-induced alopecia, providing significant psychological and physical benefit to cancer patients.

Chemotherapy-induced alopecia: how do we protect against this adverse side effect?

Chemotherapy-induced alopecia (CIA) is a common and distressing adverse effect of cancer therapy, which is known to cause severe anxiety among patients, to the extent that a proportion may refuse treatment [1]. CIA can occur following treatment with various chemotherapy drugs but is particularly prevalent following administration of cyclophosphamide, doxorubicin, daunorubicin, docetaxel, etoposide, ifosfamide, and paclitaxel. Currently, a primary treatment for CIA is the use of scalp cooling caps which, by reducing scalp perfusion, lower uptake of chemotherapeutic drugs into the hair follicles (HFs). This technique displays some level of efficacy but is not without risk (i.e., reduced drug uptake into scalp metastases) [2]. As such, CIA remains a major unmet challenge in clinical oncology and novel treatments are urgently required.

The pathobiology of CIA has recently been reviewed by Paus *et al.* [3]. Briefly, it is the highly proliferative matrix cells of anagen (actively growing) HFs (see Glossary), located in the lower bulb region (Figure 1A), that are most susceptible to damage [3], rapidly undergoing apoptosis upon exposure to chemotherapy. The slowly dividing bulge region stem cells (Figure 1A) are generally left intact, allowing hair regrowth once the chemotherapeutic insult is removed, yet this is not always the case. Indeed, CIA sometimes results in permanent hair loss, probably resulting from the destruction of epithelial hair follicle stem cells

Glossary

Anagen: during this hair cycle stage, rapidly proliferating keratinocytes of the hair matrix terminally differentiate in order to generate the hair shaft. On the scalp 80–90% of HFs are in anagen.
Arrector pili muscle: small bundles of smooth muscle attached to HFs inferior to the sebaceous gland. The insertion of the arrector pili muscle marks the anatomical location of the HF stem cell population in the outer root sheath.
Bulb region: the proliferative hair bulb contains the rapidly dividing matrix keratinocytes, which are responsible for the formation of the hair shaft and inner root sheath. The HF pigmentary unit (HFPU) is also located in this region.
Bulge region: the anatomical region of HF known to house the HF stem cell population.
Hair cycle: cyclic transformation of the HF from a large miniorgan that generates pigmented hair shafts (anagen), via a rapid involution stage (catagen) to a state of relative quiescence (telogen).
Hair follicle pigmentary unit (HFPU): early during anagen, melanocytes from the HFPU inject melanin-loaded melanosomes into keratinocytes, the basis of hair shaft pigmentation.
Isthmus: the mid region of the HF is commonly termed the isthmus. This demarcates a region below the upper HF (infundibulum), from the sebaceous gland duct to the insertion of the arrector pili muscle.
Pilosebaceous unit: composed of the HF, sebaceous gland, and arrector pili muscle.
Stem cells: HFs have multiple stem cell populations. The most important, in the context of chemotherapy-induced alopecia, are located inside the HF epithelium, namely in the bulge region of the outer root sheath. Epithelial stem cells and melanocyte stem cells in the bulge give rise to more differentiated progenitors, which eventually generate hair matrix keratinocytes or HFPU melanocytes, respectively, during anagen.

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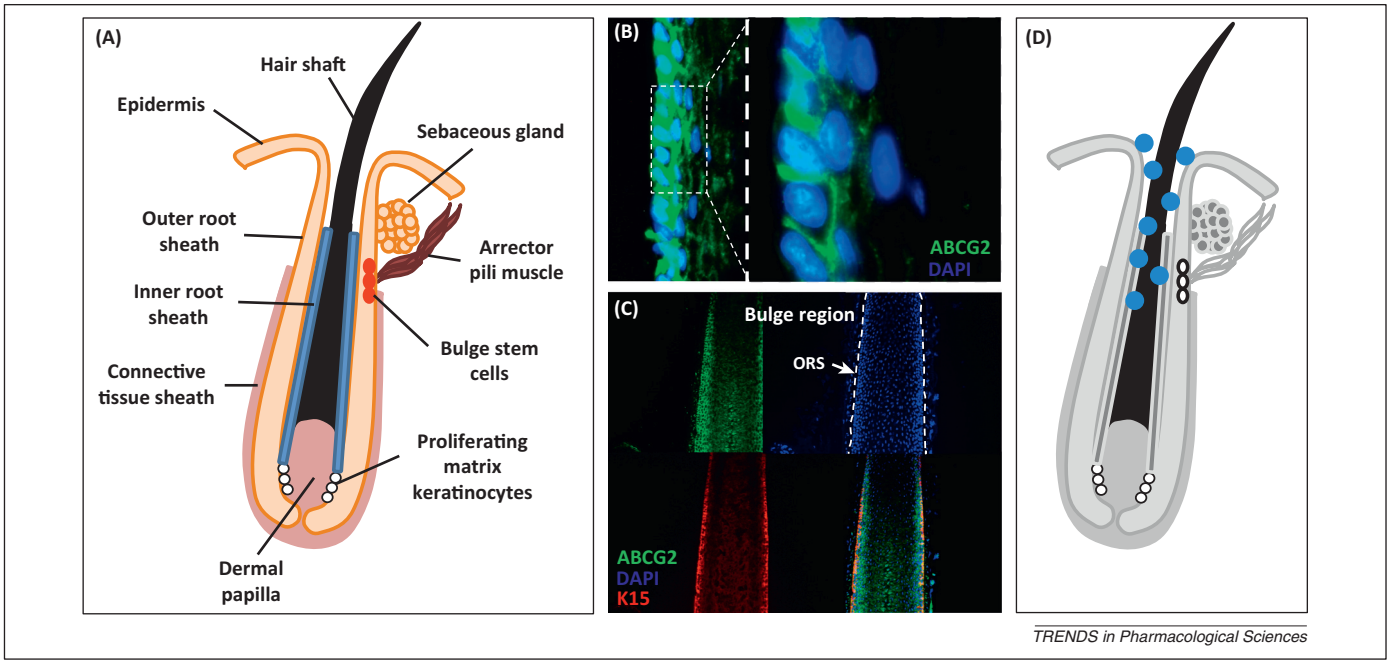

Figure 1. Morphology of the human hair follicle and expression of ABCG2. In **(A)** a cartoon depiction of a human hair follicle is displayed. Cytotoxic chemotherapy agents target the proliferative matrix keratinocyte population located in the hair bulb of anagen (actively growing) follicles. In general, the epithelial hair follicle stem cells, located at the insertion of the arrector pili muscle are undamaged; however, high dose therapies can deplete this population leading to permanent chemotherapy-induced alopecia. **(B)** ABCG2 immunoreactivity (green immunofluorescence) is confirmed in the outer root sheath (ORS) of isolated human hair follicles. **(C)** ABCG2 immunoreactivity (green immunofluorescence) colocalizes with keratin 15 (K15 – red immunofluorescence) in the bulge region (stem cell region) of human hair follicles. **(D)** Cartoon representation of a human hair follicle displaying penetration of drug-loaded nanoparticles (blue circles) into the hair canal.

(eHFSCs). Loss of these stem cells prevents the production of a new HF and, additionally, damage to melanocyte precursors also located in this region may result in altered pigmentation, should hair growth return. Permanent hair loss is increasingly associated with busulfan and cyclophosphamide pretreatment prior to bone marrow transplant [4]. Such high dose therapies appear to increase the risk of permanent alopecia and, as yet, there is no efficacious treatment.

To reduce the HF damage caused by chemotherapy, it is evident that targeting a mechanism by which HFs, and importantly their associated stem cell pools, could be protected from the toxic challenge of chemotherapy would be of significant benefit to cancer patients. Potential therapeutic targets that we believe are worthy of investigation are membrane efflux transporters of the ATP-binding cassette (ABC) family. The ABC family of transporters utilizes the energy released from ATP hydrolysis to move a wide variety of substrates, including ions, amino acids, lipids, metabolites, and drugs, across extra- and intracellular membranes. Some of these transporters fulfill a crucial role in xenobiotic defense across a range of species and within diverse tissues. Historically, research has focused on their role in the export of toxic cancer chemotherapeutics and in multidrug resistant tumors. Ultimately, with the development of ABC transporter knockout mice, their role in protecting numerous host tissues is now apparent. Given this well-recognized role in reducing the accumulation of anticancer agents, combined with the established expression of these transporters in various stem cell populations [5], we have evaluated the gene expression of these transporters in HF bulge and non-bulge keratinocytes. Based on these expression patterns and known substrate preferences (Table 1), we further speculate on the role of ABC transporters in protecting eHFSCs against chemotherapeutic insult. In addition, we propose that the prevalence of CIA might be reduced by increasing the expression of certain ABC transporters in eHFSCs. The use of specific ligands to target regulatory nuclear receptors might feasibly be used to upregulate ABC transporters, thereby preventing cytotoxic cell damage in the HF.

ABC transporters: an overview

Transporters of the ABC superfamily encompass a diverse array of membrane proteins primarily involved in the ATP-dependent export of compounds from numerous cell types [6–8]. Traditionally, certain members of the ABCB, ABCC, and ABCG subfamilies, with a few notable exceptions (e.g., cystic fibrosis transmembrane conductance regulator; CFTR; ABCC7), have been described in relation to defense against toxic insult. Many tumor types display enhanced ABC transporter expression, particularly ABCB1 (P-glycoprotein, MDR1) and ABCG2 (breast cancer resistance protein; BCRP, MXR), resulting in the reduced efficacy of chemotherapy treatments or complete resistance of the tumor to the drug therapy [9].

Compellingly, these transporters are also present in certain stem cell populations, including hematopoietic stem cells [7], cancer stem cells [8], and embryonic stem cells [10]. Although the physiological role of many of these transporters in protecting stem cells against endogenous substances is largely unknown, evidence has shown that ABCG2-expressing hematopoietic and embryonic stem cells are shielded from excessive accumulation of metabolic intermediates. Protection against toxic insult is, therefore,