Crafting and grafting of new cells to treat epidermolysis bullosa

Epidermolysis bullosa (EB) is a disease that causes the skin to become extremely fragile, which leads to the formation of blisters. Depending on the form the disease takes, ranging from relatively mild to terribly severe, the blistering and tissue erosion can occur not only on the outside of the body but on the inside as well. A severe form of epidermolysis bullosa, generalized recessive dystrophic epidermolysis bullosa (RDEB), is caused by the dysfunction of a specific gene. The mutated and nonfunctional gene in RDEB is called *COL7AI*, and its role in a normally functioning organism is to make sure sufficient type VII collagen (C7) is created in the body. C7 is a type of collagen that plays a role in ensuring that two layers of the skin, the epidermis and the dermis, remain tightly connected. In an individual with properly functioning *COL7AI*, the tight bond of the two skin layers prevents blister formation. However, when the gene is mutated, the connection between them is disrupted and blistering occurs.

Treatment of RDEB is a complex process, since the genetic mutation leads not only to problems with the skin but also with the lining of internal body structures. Due to the multifaceted nature of the disease, many different treatment methods are being researched in the hope of improving the quality of life of affected patients. One of these methods, bone marrow transplantation, has been shown to increase the production of C7 in the skin of treated patients, which improves skin integrity and decreases the amount of blistering. Additional treatment methods that are currently being explored are based on the strategy of correcting the root of the disease: the gene mutation. Gene editing targets the mutation and introduces corrected genetic material into the DNA in hopes of repairing the mutation. Despite the beautifully logical nature of this therapeutic mechanism, correcting the problem at the very root of the disease cascade, the human genetic code and the processes that follow its commands are complex, tightly regulated, and precise. This creates a requirement that its manipulation follow the same criteria.

Knowledge of inherent mechanisms of DNA breakage repair such as nonhomologous end joining (NHEJ) and homology-directed repair (HDR) allows research to utilize these reparative techniques in gene therapy. NHEJ joins two available ends of the DNA strands in order to fix a break. HDR, a less error-prone mechanism, employs a template to precisely create proper base pairing to seal the break. In gene editing the repair mechanisms are triggered by the introduction of nucleases into the "therapeutic package" sent to the mutated genetic location.

Nucleases are used to break the DNA at the site of the mutation, and a number of "site-specific" nucleases are used to ensure that this breakage occurs at the desired location. Site-specific nucleases include transcription activator-like effector nucleases (TALENs), clustered regularly interspaced short palindromic repeats (CRISPRs), and CRISPR associated 9 endonuclease systems (Cas9), each with advantages and disadvantages. For example, using a viral vector we can import into a cell site-specific nucleases packaged with the desired DNA template; the site-

specific nuclease will create a break at the desired DNA location, and HDR will be triggered to repair it using the provided DNA template.

For patients with RDEB, the desired clinical intervention is the one that will help as soon as possible. The swiftly progressing and constantly evolving research that is focused on refining the various aspects of gene therapy can ensure that gene editing becomes the therapeutic method that meets these requirements.

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