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Commentary

Reading through the nonsense: Gentamicin and ELX-02 as rescuing therapies for epidermolysis bullosa

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Nonsense mutations account for approximately 25% and 80% of genetic mutations in two life-threatening skin blistering conditions: recessive dystrophic epidermolysis bullosa (RDEB) and junctional epidermolysis bullosa (JEB), respectively. In this issue of *Molecular Therapy Nucleic Acids*, Levian et al. demonstrated that ELX-02, an aminoglycoside analog that induces readthrough of premature termination codons (PTCs), restored the expression and function of the missing proteins in RDEB and JEB patient-derived keratinocytes/fibroblasts, as well as their three-dimensional (3D) organotypic skin equivalents.[1]

RDEB arises from mutations that impair collagen VII (C7), a protein crucial for forming anchoring fibers that connect the dermis to the epidermis in the basement membrane zone. Patients with RDEB develop extensive blistering of the skin, oral mucosa, and gastrointestinal and genitourinary tracts. Chronic blistering leads to complications arising from wounds and fibrotic scars that include deformation of the hands (pseudosyndactyly) and development of an aggressive form of squamous cell carcinoma.[2] JEB stems from mutations that disrupt proteins essential for forming the lamina lucida, a key layer of the basement membrane needed to attach the lamina densa and dermis to the epidermis. The most severe form of JEB arises from mutations in laminin-332, a critical protein in maintaining the lamina lucida of the basement membrane.[2] The mean life expectancy of this condition is only 6.5 months, according to a study of 66 infants born with severe JEB.[3] While various therapeutic strategies, including stem cell therapies, protein therapy, and gene therapies, are under extensive investigation, there is an urgent unmet medical need since current treatment has been mainly limited to supportive care for wound healing and prevention of infection.

Aminoglycosides, such as gentamicin, are typically used as antibiotics to treat bacterial infections by disrupting protein translation through binding of the 30S ribosomal subunit. It was subsequently discovered that aminoglycosides possess the ability to interact with the ribosomal acceptor site, inhibit stop codon recognition, and promote PTC readthrough (Figure 1). Based on this concept, groups led by Drs. David T Woodley and Mei Chen conducted short-term clinical trials using gentamicin to promote PTC readthrough and restore essential protein function to improve the quality of life for individuals with RDEB or JEB resulting from nonsense mutations. In these studies, patients were given gentamicin topically for 2 weeks, intradermally for 2 days, or intravenously for 14–24 days.[4–6] In all these clinical studies, new expression of C7 or laminin-332 was observed at dermal-epidermal junctions, and wound healing was improved, with no reported adverse effects.

Unfortunately, long-term use of aminoglycosides has been associated with nephrotoxicity and ototoxicity due to their off-target interaction with mitochondrial ribosomes. ELX-02, a novel small-molecule and non-antibiotic aminoglycoside analog, was developed as a safer alternative for PTC readthrough treatment, aiming to reduce associated toxicity. It has been demonstrated to have low affinity to either prokaryote ribosomes (thus no anti-bacterial activity) or eukaryotic mitochondrial ribosomes (thus less toxicity) while maintaining or increasing the affinity toward eukaryotic ribosomes in the cytosol (high specificity). In a recent study by Levian et al. and from the same research groups performing gentamicin clinical trials in patients with EB, ELX-02 was used to treat cells derived from patients with RDEB and JEB arising from nonsense mutations. Their study showed that ELX-02 dose-dependently induced C7 and laminin β3 (a subunit of laminin-332) expression in patient-derived primary keratinocytes and fibroblasts. Excitingly, ELX-02 treatment induced higher C7 and laminin β3 levels than gentamicin at significantly lower doses, even surpassing those of normal control cell lines in some cases. The C7 and laminin β3 expression rescued by ELX-02 properly localized to the dermal-epidermal junctions, demonstrated by 3D skin equivalents. As a result, cellular dysfunctions, such as hypermotility and poor adhesion, were also rescued by ELX-02, likely via restoration of basement membrane integrity.

While ELX-02 has not yet been clinically investigated in patients with RDEB or JEB, both single and multiple dose-escalation studies involving healthy subjects,[7,8] as well as patients with renal impairment,[9] nephropathic cystinosis, and cystic fibrosis, have been conducted. While these studies have proven ELX-02 to be generally safe and well tolerated, its clinical efficacy has yet to be determined. Moreover, these were short-term studies that limited the route of administration to subcutaneous injections, leaving its long-term and systemic use uninvestigated. Since RDEB and JEB are lifelong

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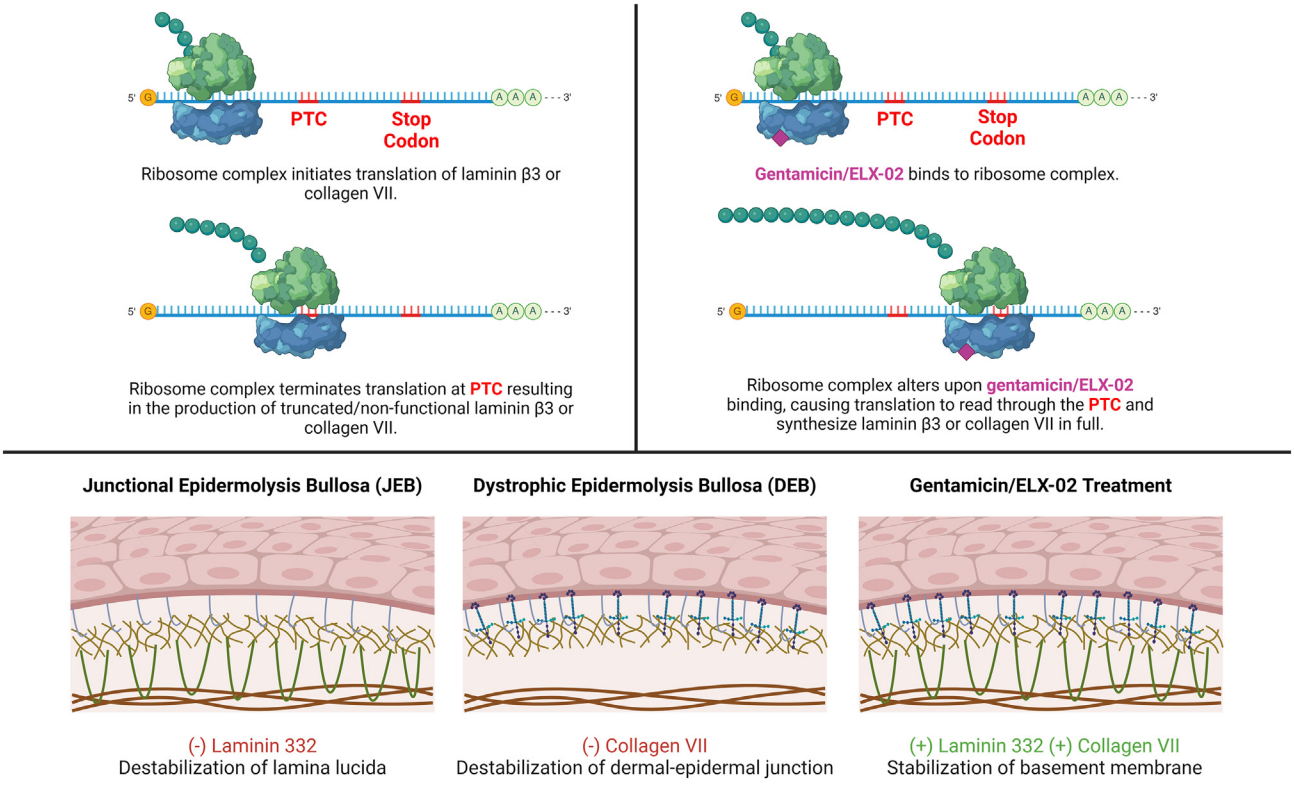

Figure 1. Proposed mechanism of PTC readthrough therapy to rescue basement membrane function in RDEB and JEB

Gentamicin and ELX-02 enable translation past premature termination codons (PTCs), which cause disfunction of C7 or laminin-332 underlying the pathogenesis of RDEB and JEB, respectively. Restoration of basement membrane integrity strengthens the connections of the dermis to the epidermis, which resolves skin fragility.

conditions, evaluating the long-term safety and efficacy of gentamicin and ELX-02 is essential. Animal models would enable extended treatment and detailed toxicity analysis, which is not possible in human trials. Unfortunately, current animal models of RDEB and JEB lack the nonsense mutations treatable by these drugs. Considering the potential life-changing impact of these treatments, developing animal models of these conditions with nonsense mutations would be pivotal for advancing research.

Although both gentamicin and ELX-02 represent a much-needed route of treatment for both patients with RDEB and those with JEB, their mechanism of action requires a high specificity in diagnosis. Due to the lower frequency of PTC-associated mutations in COL7A1, it would be advisable to test patients with RDEB for relevant nonsense mutations before prescribing these medications. On the other hand, given the large proportion of patients with JEB (>80%) harboring nonsense mutations, their condition severity, early lethality, and lack of effective treatments, using PTC readthrough therapy may prove to be vital immediately upon diagnosis with severe JEB. Despite their limitations, we believe these therapies represent personalized and evidence-based approaches that will characterize the future of medical practice.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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