

Wound Repair Capability in EDS Fibroblasts can be Retrieved by Exogenous Type V Collagen

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Ehlers-Danlos syndrome (EDS) refers to a group of rare, inherited, connective tissue disorders affecting joints, skin, and blood vessels[1]. The most common type, according to the current clinical classification, is Classical EDS, comprising the former EDS type I (OMIM 130000; <http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=130000>) and type II (OMIM 130010; <http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=130010>) characterized by joint hypermobility, skin hyperextensibility, and impaired wound healing with abnormal scars. In Classical EDS, mutations affecting type V collagen genes have been found and the most common mutations lead to a nonfunctional COL5A1 allele[1]. Type V collagen, a minor component of extracellular matrix (ECM), is essential for type I collagen fibril formation, acting as nucleator and regulator of fibril diameter[2,3], and plays a fundamental role in the development of functional connective tissues. Mutations in type V collagen caused *in vitro* the deposition of a defective fibrillar network of collagens and fibronectin associated to an altered pattern of integrins[4,5]. Since both integrins and fibronectin have a pivotal role in wound repair[6], the impaired wound healing observed in Classical EDS could therefore be explained by the poor organization of type V collagen-rich connective tissues, such as skin.

On these bases, a very recent paper[7] investigated *in vitro* wound repair in fibroblasts derived from Classical EDS patients with type V collagen deficiency and a markedly delayed wound healing, in order to evaluate their repair capability and their adequacy as tools for testing the effect of potential repairing therapeutics. In this study, fibroblasts deposited, as expected, a defective ECM lacking fibrillar networks of collagens and fibronectin, and showed a down-regulation of $\alpha_5\beta_1$ and $\alpha_2\beta_1$ integrins, binding collagen and fibronectin, respectively, while $\alpha_v\beta_3$ integrin, an auxiliary receptor for fibronectin, was up-regulated. The authors designed an *in vitro* wounding assay where mutant fibroblasts were unable to move into a previously scratched area, which simulates a wound, showing a marked delay in repair (Fig. 1a). It is interesting to note that, immediately after scraping, the border between wound and confluent monolayer was sharp in control fibroblasts, whereas in EDS cells, it was not so evident and cells appeared partly detached far beyond the scraping line. After 48 h, control cells had uniformly migrated into the acellular

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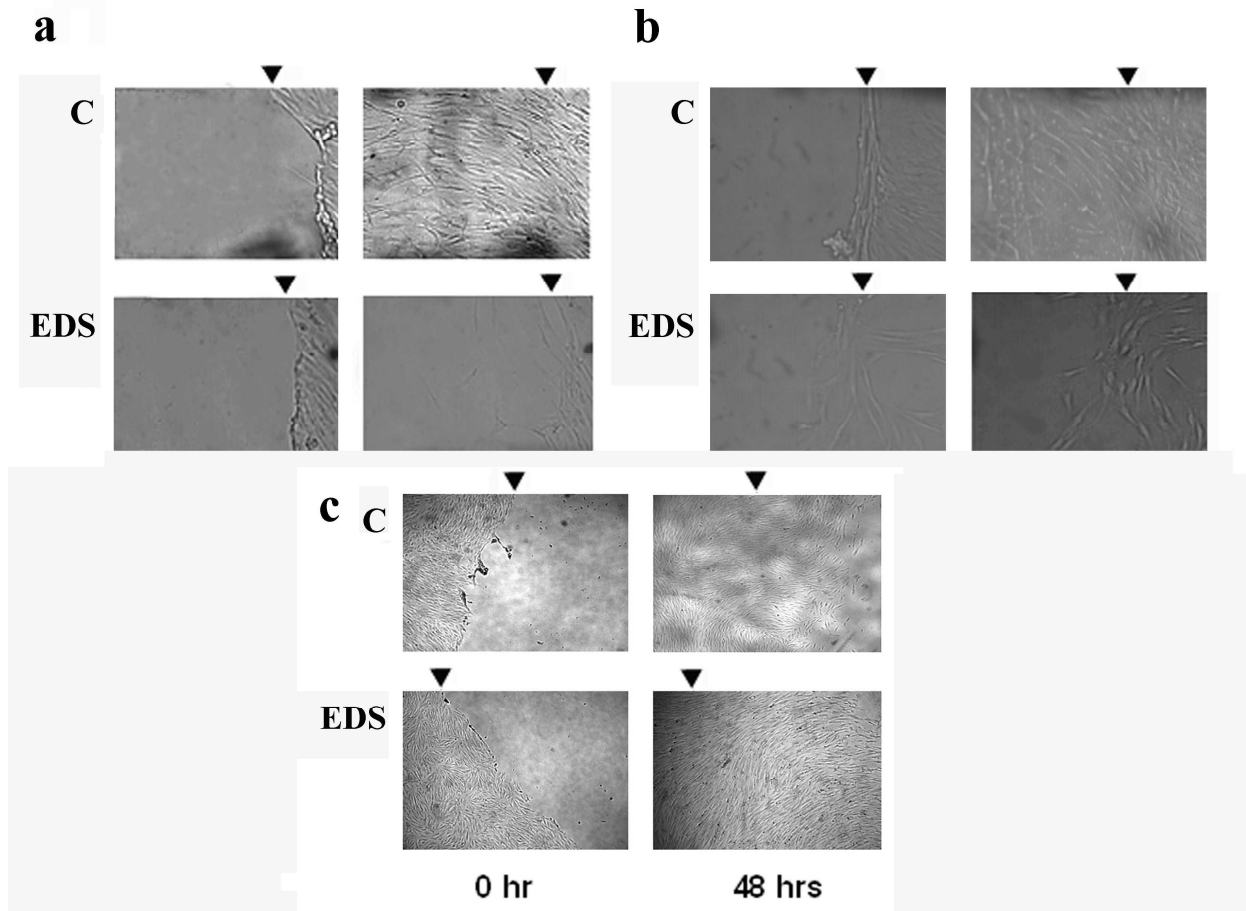


FIGURE 1. Wound healing and migration in EDS fibroblasts. (a) Fibroblasts of control and Classical EDS patients were scraped (0 h) and allowed to migrate for 48 h (48 h). (b) Fibroblasts were scraped as above and allowed to migrate in the presence of IGFBP-1. (c) Fibroblasts were grown in medium supplemented with type V collagen. After wounding, fibroblasts were allowed to migrate in the same supplemented medium. Arrowheads indicate the wound edge.

area, whereas EDS cells were still very sparse and even more disorganized than immediately after scraping. In the attempt to improve scratch repair, the authors tested insulin-like growth factor-binding protein-1 (IGFBP-1), known to improve wound healing[8] through signaling via $\alpha_5\beta_1$ integrin, and type V collagen, capable of restoring ECM organization when added to cultured EDS fibroblasts[5]. IGFBP-1 stimulated the migration and scratch repair of control fibroblasts, but not that of EDS strains (Fig. 1b); their abnormal integrin pattern inhibited its positive effect on cell migration. The migratory capability, instead, remarkably improved in the presence of exogenous type V collagen (Fig. 1c); the scraping line in EDS fibroblasts was sharper and they migrated as the controls. The authors concluded that the delayed scrape repair in EDS fibroblasts is caused by the concurrence of impaired fibrillar network and altered integrin pattern. The choice of therapeutics meant for improvement of wound repair in EDS cells should therefore consider the possible involvement of integrins in the cellular response.

Normal wound healing is a highly regulated process and requires several factors. Fibrillar matrix, fibronectin, and its $\alpha_5\beta_1$ integrin receptor allow fibroblast migration and adhesion. Additional ECM molecules, acting as regulators and organizers, are necessary as well for the maintenance of fibrillar matrix functions and integrity. Wound healing impairment can therefore be the consequence of mutations affecting different genes. Murine models, lacking the transmembrane heparan sulfate proteoglycan syndecan-4, a modulator of integrin activity, show significant delayed healing of skin wounds and impaired angiogenesis[9]. In a more recent study[10], it has been demonstrated that *in vitro*-delayed

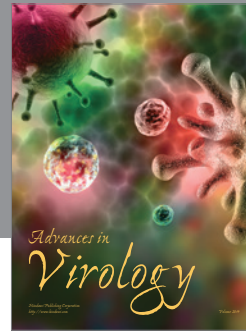
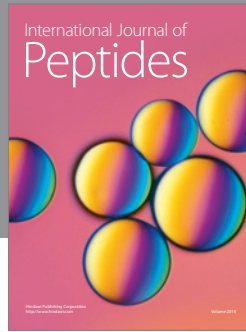
scrape repair in a progeroid form of EDS was associated to a β 4GalT-7 gene mutation affecting the biosynthesis of heparan sulfate. Mutations in β 4GalT-7 and tenascin-X[11] genes suggest that the extreme clinical heterogeneity in EDS likely derives from genetic heterogeneity. The search for causative mutations must therefore be addressed not only to collagen genes or to genes coding for collagen modifying enzymes, but also to genes coding for nucleators/organizers of the ECM. Complex EDS pathogenesis may be understood only through the knowledge of the fine regulation determining ECM complex architecture.

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