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Pigment epithelial-derived factor: a new player in the calcification of dermal elastic fibre?

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Dear Editor, Pigment epithelium-derived factor (PEDF) is an endogenously produced glycoprotein expressed in several organs during developmental stages and adulthood and mainly acting on cell differentiation. In vitro and in vivo studies have demonstrated that PEDF has neurotrophic and antioxidant characteristics as well as the ability to counteract angiogenesis, tumorigenesis, atherosclerosis, thrombosis and inflammation. In addition, PEDF has been also related to bone metabolism, increasing the expression of alkaline phosphatase and promoting osteoblast differentiation.

To our knowledge, there is little information on PEDF expression and activity in dermal physiopathology. Moreover there are no data on the expression and localization of PEDF in skin ectopic calcification. Therefore, we examined in vitro both PEDF expression and alkaline phosphatase activity in adult human dermal fibroblasts (from five healthy donors aged 30–40 years) cultured in standard [Dulbecco’s modified Eagle’s medium (DMEM)] or in calcifying medium. All samples used in the present study were obtained after the participants gave their informed consent, according to the guidelines of the Declaration of Helsinki and of the ethics committee of the local school of medicine. After 20 days of culture, when a mineral deposit was present on the cells maintained in the calcifying medium (Fig. 1b), the fibroblasts exhibited an increased expression of PEDF (Fig. 1b, insert) and enhanced alkaline phosphatase activity (Fig. 1c), compared with cells in DMEM (Fig. 1a, insert, c). These data confirm previous in vitro observations in bone stem cells, supporting the hypothesis that PEDF also contributes to the calcification process in soft connective tissues.

In order to exclude the possibility that these effects might have been due to the artificial calcifying environment of the culture system, thus limiting the in vivo role of this glycoprotein, we then investigated the expression and localization of PEDF in the dermis of patients affected by pseudoxanthoma elasticum, a rare disease characterized by the aberrant mineralization of elastic fibres of soft connective tissue in specific areas and by fibroblasts exhibiting in vitro a procalcifying signature and increased alkaline phosphatase activity.

Skin biopsies (3–4 mm in diameter including papillary and reticular dermis, fixed and embedded in Spurr’s resin) were obtained from healthy participants (n = 5) and from patients with pseudoxanthoma elasticum (n = 6), all 30–40 years of age, for morphological evaluation. As pseudoxanthoma elasticum is more frequently observed in women than men, we analysed samples derived from women. Clinical diagnosis was confirmed by biomolecular analyses demonstrating causative mutations on the ABCC6 gene.

The severity of the pathological phenotype was quantified according to the Phenodex index, which nowadays represents the only published standardized system quantifying the clinical...
manifestations of pseudoxanthoma elasticum. A score of S1 was given to skin with papules or bumps (Fig. 2b); a score of S2 to plaques of coalesced papules and a score of S3 to lax and redundant skin (Fig. 2c). In this research we studied patients with scores of S1 or S3. PEDF expression was investigated by immunohistochemistry, allowing the ex vivo evaluation of the architecture and morphology of preserved tissue.

PEDF was localized in all keratinocytes of both normal and pseudoxanthoma elasticum skin (Fig. 2d–f). In contrast, mesenchymal cells (i.e. fibroblasts and endothelial cells) independent of the disease were strongly stained in the papillary dermis, whereas positivity was negligible in most cells of the reticular dermis (Fig. 2j). These data are in agreement with the association between PEDF expression and the potential to replicate cells and the different growth capabilities of fibroblasts from different dermal areas.

Consistently with the observation that PEDF binds to collagens type I and III, the extracellular matrix exhibited a uniform positivity of collagen bundles in the papillary and the reticular dermis of healthy participants (Fig. 2d, g). Independent of the pathological condition, comparable results were also observed in pseudoxanthoma elasticum biopsies (Fig. 2 e, f, h, i).

A moderate positivity comparable with that of collagen was observed on the normal elastic component of healthy participants (Fig. 2g) and on unaffected elastic fibres of patients with pseudoxanthoma elasticum (Fig. 2j). On the contrary, mineralized elastic fibres exhibited a remarkably strong positivity for PEDF (Fig. 2h, i, j). The extent of immune staining appeared to be proportional to the amount of calcified elastic fibres and consequently to the severity of dermal alterations categorized according to the Phenodex score (i.e. S1 and S3) (Fig. 2b, c).

As glycosaminoglycans are known to be abnormally secreted by pseudoxanthoma elasticum fibroblasts and to be localized on calcified elastic fibres and on the electron-dense material associated with these fibres, it is conceivable that glycosaminoglycans can contribute to the accumulation of PEDF due to a high binding affinity between these molecules. Despite the known antiangiogenic effects of PEDF, the increased amount of this glycoprotein has recently been associated with enhanced vascular endothelial growth factor and mineral deposit, suggesting that the effects of PEDF depend on the cell type (i.e. mesenchymal stem cells vs. other cells). This finding is consistent with increased vascular endothelial growth factor and the hydroxyapatite deposits found in pseudoxanthoma elasticum.

A limitation of this study may be the relative small number of samples. However, pseudoxanthoma elasticum is a rare disease and, therefore, large-scale studies appear unrealistic. This is the first evidence of the occurrence of PEDF in dermal
ectopic calcification. Future studies may confirm whether PEDF can become a potential target of therapeutic perspectives in counteracting the pathological mineralization of soft connective tissues.

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