Research letter

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

DOI: 10.1111/bjd.15223

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.¹ *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.^{1,2} In addition, PEDF has been also related to bone metabolism, increasing the expression of alkaline phosphatase and promoting osteoblast differentiation.³

To our knowledge, there is little information on PEDF expression and activity in dermal physiopathology.^{4,5} 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,³ 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.⁶

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.⁷ 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

Fig. 1. Fibroblasts cultured in standard Dulbecco's modified Eagle's medium (DMEM) or in calcifying medium (CM) for 20 days. (a–b) The mineral deposit visualized by von Kossa staining (arrows) was assessed on dermal fibroblasts cultured in DMEM (a) and in a calcifying medium. (b) A representative Western blot for pigment epithelium-derived factor is shown in the inserts. To allow the normalization of protein expression, an equal amount of protein (sample of 40 µg) was loaded on polyacrylamide gel for electrophoresis. SERPINF1 antibody (GenWay Biotech Inc., San Diego, CA, U.S.A.) was used at 1 : 1000 dilution. (c) Alkaline phosphatase (ALP) activity per milligram of protein is expressed as box and whisker plots. The horizontal line shows the median value for each condition and the boxes represent the interquartile range, indicating the full extent of the minimum and maximum values. *** $P < 0.001$.

Fig. 2. Pseudoxanthoma elasticum, clinical and immunohistochemical findings. (a) Normal skin. (b) Typical skin papules in a patient with a mild dermal phenotype (Phenodex score S1). (c) Severe dermal alterations represented by lax and redundant skin (type S3). (d–f) Pigment epithelium-derived factor (PEDF) immune staining of the epidermis and papillary dermis of (d) healthy participants and patients with a score of (e) S1 and (f) S3. (g) Moderate PEDF immune staining of normal matrix constituents in the reticular dermis [collagen (C) and elastic fibres (arrows)]. (h, i) Strong PEDF immune staining on calcified elastic fibres (arrowheads) in the reticular dermis of patients with a score of S1 and S3. (j) Whole skin thickness of a patient with an S1 score, clearly exhibiting the strong positivity of the epidermis and mesenchymal cells in the papillary dermis and calcified elastic fibres (arrowheads). Collagen bundles (C) and normal elastic fibres (arrows) show a moderate positivity, comparable to controls. SERPINF1 antibody was used at 1 : 50 dilution using the ultraview universal 3'-3'-diaminobenzidine hydrochloride kit in an automated immunohistochemistry in situ hybridization slide staining system BenchMark XT.

manifestations of pseudoxanthoma elasticum.⁸ 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 cells⁵ and the different growth capabilities of fibroblasts from different dermal areas.⁹

Consistently with the observation that PEDF binds to collagens type I and III,¹⁰ 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. 2e, 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).⁸ 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,¹¹ it is conceivable that glycosaminoglycans can contribute to the accumulation of PEDF due to a high binding affinity between these molecules.¹² Despite the known antiangiogenic effects of PEDF,² 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).¹³ This finding is consistent with increased vascular endothelial growth factor¹⁴ and the hydroxyapatite deposits found in pseudoxanthoma elasticum.⁶

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.

Department of Life Sciences, University of
Modena and Reggio Emilia, Via Campi
287–41125, Modena, Italy
Correspondence: Daniela Quaglino.
E-mail: daniela.quaglino@unimore.it

F. BORALDI
L. LOSI
D. QUAGLINO

References

- 1 He X, Cheng R, Benyajati S et al. PEDF and its roles in physiological and pathological conditions: implication in diabetic and hypoxia-induced angiogenic diseases. *Clin. Sci. (Lond)* 2015; **128**:805–23.
- 2 Rychli K, Huber K, Wojta J. Pigment epithelium-derived factor (PEDF) as a therapeutic target in cardiovascular disease. *Expert Opin Ther Targets* 2009; **13**:1295–1302.
- 3 Li F, Song N, Tombran-Tink J et al. Pigment epithelium-derived factor enhances differentiation and mineral deposition of human mesenchymal stem cells. *Stem Cells* 2013; **31**:2714–23.
- 4 Abe R, Yamagishi S, Fujita Y et al. Topical application of anti-angiogenic peptides based on pigment epithelium-derived factor can improve psoriasis. *J Dermatol Sci* 2010; **57**:183–91.
- 5 Francis MK, Appel S, Meyer C et al. Loss of EPC-1/PEDF expression during skin aging in vivo. *J Invest Dermatol* 2004; **122**:1096–105.
- 6 Ronchetti I, Boraldi F, Annovi G et al. Fibroblast involvement in soft connective tissue calcification. *Front Genet* 2013; **4**:22.
- 7 Boraldi F, Garcia-Fernandez M, Paolinelli-Devincenzi C et al. Ectopic calcification in β -thalassemia patients is associated with increased oxidative stress and lower MGP carboxylation. *Biochim Biophys Acta* 2013; **1832**:2077–84.
- 8 Pfendner EG, Vanakker OM, Terry SF et al. Mutation detection in the ABCC6 gene and genotype-phenotype analysis in a large international case series affected by pseudoxanthoma elasticum. *J Med Genet* 2007; **44**:621–8.
- 9 Sorrell JM, Caplan AI. Fibroblast heterogeneity: more than skin deep. *J Cell Sci* 2004; **117**:667–75.
- 10 Kozaki K, Miyaishi O, Koiwai O et al. Isolation, purification, and characterization of a collagen-associated serpin, caspin, produced by murine colon adenocarcinoma cells. *J Biol Chem* 1998; **273**:15125–30.
- 11 Tiozzo Costa R, Baccarani Contri M, Cingi MR et al. Pseudoxanthoma elasticum (PXE): ultrastructural and biochemical study on proteoglycan and proteoglycan-associated material produced by skin fibroblasts in vitro. *Coll Relat Res* 1988; **8**:49–64.
- 12 Alberdi E, Hyde CC, Becerra SP. Pigment epithelium-derived factor (PEDF) binds to glycosaminoglycans: analysis of the binding site. *Biochemistry* 1998; **37**:10643–52.
- 13 Li F, Armstrong GB, Tombran-Tink J et al. Pigment epithelium derived factor upregulates expression of vascular endothelial growth factor by human mesenchymal stem cells: possible role in PEDF regulated matrix mineralization. *Biochem Biophys Res Commun* 2016; **478**:1106–10.
- 14 Boraldi F, Bartolomeo A, De Biasi S et al. Innovative flow cytometry allows accurate identification of rare circulating cells involved in endothelial dysfunction. *PLOS ONE* 2016; **11**:e0160153.

Funding sources: This work was supported in part by grant 2015.0306 to F.B from Fondazione Cassa di Risparmio di Modena and by grant E91J13000450007 to D.Q. from PXE Italia Onlus.

Conflicts of interest: none disclosed.