Development and characterization of two polyclonal antibodies directed against human periostin.

Vergnaud P[1], Pagnon-Minot A[2], Bertholon C[3], Lhoste Y[1], Chassaing E[1], Borel O[3,4], Gineyts E[3], Schubert T[1], Clézardin P[3], Chapurlat R[3,4], Rousseau JCh[3]

[1]Bioclinica, Lyon, France; [2]Novotec, Lyon, France; [3]INSERM Unit 1033 and University of Lyon, Lyon, France; [4]Hospices Civils de Lyon, Lyon, France

INTRODUCTION

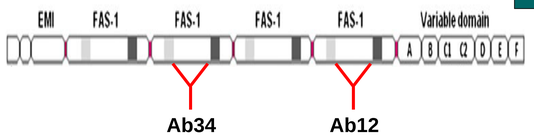
Matricellular protein periostin (PN) is mainly expressed in the periosteum. PN regulates Notch1 signaling, activates lysyl-oxydase and mediates cancer cell adhesion, survival and invasion in different tissues. The aim of this study was to develop two polyclonal antibodies against the Fas-1 region of human PN and determine their specificity in human serum and bone tissue specimens.

METHODS

In silico analysis of the total protein sequence of human PN led us to identify two amino acid sequences that are suitable for antibody generation **(Patent n° 13/375870)** in the second and fourth Fasciclin-like domains [**ETLEGNTIEIGCDGDSI** (**Ab-34)** and **KGFEPGVTNILKTTQGSK (Ab-12)**, respectively]. These two peptides were injected in rabbits following a standard protocol of 10 weeks of immunization. Specific polyclonal antibodies Ab12 and Ab34 were purified by immunoaffinity chromatography. We assessed the specificity of these antibodies for PN by immunohistochemistry (IHC) and western blotting (WB) using, respectively, human bone tissue and human sera previously depleted for albumin, IgG and transferrin.

RESULTS

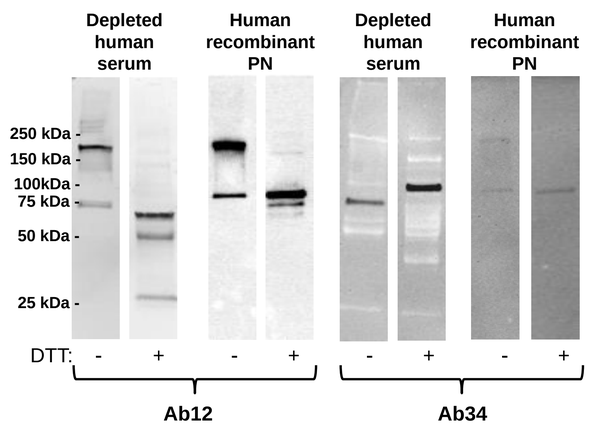
A



Localization of antibody epitopes (Patent n° 13/375870).

- **Ab12:** Rabbit-polyclonal antibody against ^{575}**KGFEPGVTNILKTTQGS**^{592} sequence of human PN.
- **Ab34:** Rabbit-polyclonal antibody against ^{322}**ETLEGNTIEIGCDGDSI**^{338} sequence of human PN.

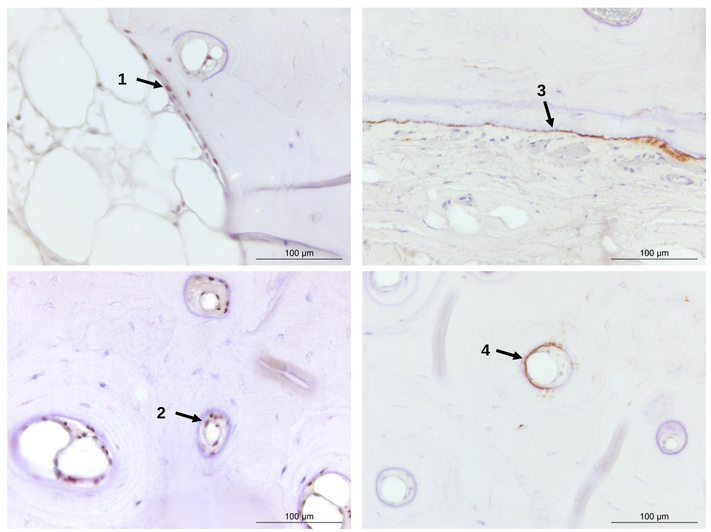
B



WB analysis with Ab12 and Ab34 antibodies.

- **Ab12** antibody recognizes two bands around 200 and 75 kDa in serum.
- After treatment by dithiothreitol (DTT) to reduce disulfide bonds, these bands disappear and 3 new bands are detected at 70, 50 and 25 kDa.
- **Ab34** recognizes a single band at 75 kDa in the serum.
- After DTT reduction, this band migrates at a molecular weight of 100 kDa, indicating that the reduction of intra-disulfide bonds in PN leads to a more extended conformation of the molecule and makes it run more slowly in the gel.
- In SDS-Page gels the recognition of human recombinant PN is better with Ab12 than Ab34 antibodies.

C



IHC of human bone with Ab12 and Ab34 antibodies.

- **Ab12** antibody shows a cytoplasmic staining only. This labeling is present in osteoblasts lining cortical bone **(1)** and in those present in the Haversian canals **(2)**.
- **Ab34** antibody shows a matrix staining of the periosteum **(3)** and of the edge of Haversian canal **(4)**.

CONCLUSION

We have generated two polyclonal antibodies against the Fas-like domains of human PN. Our preliminary results suggest that they bind at least two forms of PN molecules in the serum and bone.