Active molecules in regenerative medicine

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Background

Biomaterials play central roles in modern strategies in regenerative medicine and tissue engineering as designable biophysical and biochemical environments that direct cellular behavior and function. New generations of synthetic biomaterials are being developed at a rapid pace for use as three-dimensional extracellular microenvironments to mimic the regulatory characteristics of natural extracellular matrices (ECMs) and ECM-bound growth factors, both for therapeutic applications and basic biological studies. The ultimate decision of a cell to differentiate, proliferate, migrate, apoptosis or perform other specific functions is a coordinated response to the molecular interactions with these ECM effectors. The flow of information between cells and their ECM is highly bidirectional, as, for example, observed in processes involving ECM degradation and remodeling.

Matrix metalloproteinases (MMP) are Zn and Ca dependent enzymes and represent a class of structural and functional kindred enzymes that are involved in altering the natural compounds of the extracellular matrix [1,2]. MMP are synthesized as zymogens that can be inhibited by 4 classes of natural inhibitors called TIMPs (tissue inhibitor for matrix metalloproteinases) [3]. MMP plays important roles in physiological processes, but their overexpression plays also crucial roles in pathological processes as multiple sclerosis, arthritis, Alzheimer disease and especially in cancer and metastasis [4-8]. Among these biological molecules we have investigated expression of metalloproteinase 8 (MMP-8) and metalloproteinase 9 (MMP-9) in the periimplantar tissues remodelling.

Methodology

We have performed an indirect immunohistochemical technique using hematoxilin/eosin (HE) staining by peroxidase induced conversion of DAB (3,3-diaminobenzidine), an enzyme conjugate, which reveals the localisation of antibody-bound antigenic sites by means of a coloured reaction. DAB, a chromogenic substrate for peroxidase, stains the antigen-antibody sites in brown, indicating the presence of the metalloproteinases-8 (MMP-8) in the cells surrounding the implant. MMP-8 are usually present in the neutrophils infiltrating the connective tissue or bone in an accute phase of inflamatory process. Data analysis was done using an Olympus BX40 optical microscope with a color CCD camera mounted on. We have also performed molecular docking and molecular dynamics investigations regarding simulations of the catalytic

domain of a known matrix metalloproteinase (MMP), in the absence of the substrate or a known inhibitor, starting from ProteinDataBank published data (ID-1QIB). This study emphasizes the role of the atomic position in this site regarding to further simulations for conceiving a rather modulating inhibitor for these enzymes.

Results and conclusions

Immunolabelling with MMP-8 is reduced and limited to the perivascular area. MMP-9 has a more diffuse presence, without precise localization and therefore shows no remodeling processes (1st and 2ndtype). After 2 months MMP-8 immunolabelling was more pronounced outside blood vessel walls as a proof of chronic inflammation. MMP-9 was found perivascular (in the wall of small capillaries) more diffuse but also more intense. This suggests a lot of active remodeling here (3rd and 4th type). For 2nd type MMP-9 is present in a diffuse but more intense way suggesting accelerated tissue remodeling. These preliminary phenomenon modulated by the presented molecules are crucial for newly grown bone tissue formation and healing, even allowing implant primary fixation. Molecular modelind and dynamic studies on MMP2 catalytic site showed that Root Mean Square Fluctuation (RMSF) was performed, compared with the NMR starting structure and the hydrophobic solvent accessible surface area (HSAS) of the MMP2 catalytic site. We have observed small fluctuations for the catalytic Zn area (high stability); however we have noticed some fluctuations, especially in some loop regions near the catalytic site of the MMP, in 192-250 region of the MMP2 PDB file. This study emphasizes the role of the atomic position in the catalytic site for MMPs, regarding to further simulations for conceiving a rather modulating inhibitor for these enzymes.

References

1. Shapiro SD. "A concise yet informative stroll through matrix metalloproteinases and TIMPs." J.Cell.Sci., no. 113, vol.19, 2000, pp.3355-3356.
2. Woessner JF Jr. "Matrix metalloproteinase inhibition. From the Jurassic to the third millennium". Ann N Y Acad Sci. vol.30, no.878, 1999, pp.388-403.
3. Brew K, Dinakarpandian D, Nagase H, "Tissue inhibitors of metalloproteinases: evolution, structure and function." Biochim Biophys Acta, vol.1477, 2000, pp.1-2.
4. Fang L, Huber-Abel F, Teuchert M, Hendrich C, Dorst J, Schattauer D, Zettlmeissel H, Wlaschek M, Scharffetter-Kochanek K, Tumani H, Ludolph AC, Brettschneider J. "Linking neuron and skin: Matrix metalloproteinases in amyotrophic lateral sclerosis (ALS)". J Neurol Sci. 2009, epub.
5. Ribeiro BF, Iglesias DP, Nascimento GJ, Galvão HC, Medeiros AM, Freitas RA. "Immunoexpression of MMPs-1, -2, and -9 in ameloblastoma and odontogenic adenomatoid tumor." Oral Dis. 2009, epub.
6. Korpos E, Wu C, Sorokin L. "Multiple roles of the extracellular matrix in inflammation." Curr Pharm Des. Vol.15 no.12, 2009, pp. 1349-57.
7. Ando A, Hagiwara Y, Tsuchiya M, Onoda Y, Suda H, Chimoto E, Itoi E. "Increased expression of metalloproteinase-8 and -13 on articular cartilage in a rat immobilized knee model". Tohoku J Exp Med. No.217, vol 4, 2009, pp.27127-8
8. Gentner B, Wein A, Croner RS, Zeittraeger I, Wirtz RM, Roedel F, Dimmler A, Dorlaque L, Hohenberger W, Hahn EG, Brueckl WM. "Differences in the gene expression profile of matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) in primary colorectal tumors and their synchronous liver metastases. Anticancer Res. no.29, vol.1, 2009, pp.67-74.