Activity of monomeric Smac mimetic compound on human fibroblast like synoviocytes from rheumatoid arthritis patients

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Rheumatoid arthritis (RA) is a chronic polyarticular disorder and the rheumatoid synovium harbours a special cell population, known as fibroblast like synoviocytes (FLS) (1). The FLS produce cytokines and matrix-degrading enzymes that mediate the interaction with inflammatory and endothelial cells and are responsible for the progressive destruction of articular cartilage and bone. In this scenario, the production of cytokines and chemokines within the rheumatoid synovium would help to recruit T cells, macrophages and neutrophils, which, in turn, attract more inflammatory cells and enhance the activated state of the FLS and of osteoclasts (2).Deficient apoptosis and, thus, prolonged survival of FLS results from up-regulated anti-apoptotic molecules (IAP) at sites of synovial invasion into cartilage and bone (3). The anti-apoptotic activity of IAP proteins can be negated by the mitochondrial protein Smac (second mitochondrial activator of caspases) which is liberated into the cytoplasm in response to pro-apoptotic stimuli (4).The aim of this study was to investigate the pro apoptotic activity of Smac 127 in FLS from patients with RA, because this molecule could represent a new therapeutic approach for patients who are resistant to classical therapy or in alternative to classical medicines. The human FLS have been isolated by synovial tissues of patient suffering from active arthritis rheumatoid. The preliminary study of apoptosis induction by Smac 127 on FLS was performed with the annexin V test and the flow cytometry analysis demonstrated that Smac 127 induce a significative apoptosis in FLS of all patients analysed. This apoptotic activity was induced by a down regulation of IAPs consequently we evaluated the levels of cIAP1, cIAP2 and XIAP on FLS and OA extracts treated with monomeric Smac 127 compared with Smac 066 (control). Smac 127 was able to reduce the level of IAPs. The caspase activation, induced by this molecule, was confirmed by the appearance of active caspase-8 and caspase-3. Furthermore the downregulation of IAPs protein involved many proteins of the apoptotic pathway, we investigated the apoptotic pattern of FLS treated with Smac 127 by apoptotic array. Furthermore IGFBP-5, known to be involved in proliferation, differentiation, apoptosis and osteoblastic activation (5) was significantly upregulated by SMAC 127. We found this result particularly interesting and the involvement of IGFBP5 in osteoblast genesis will be the subject of our future study.

1. Marrack P, Kappler J, Kotzin BL. Autoimmune disease: why and where it occurs. Nat Med 2001;7:899–905.
2. Tarner IH, Muller-Ladner U, Gay RE, Fathman CG, Gay S. The Pathogenesis of Rheumatoid Arthritis – Gene Transfer to Detect Novel Targets for Treatment. Frontiers in Autoimmunity 2003; 315–346.
3. Vaux DL, Silke J. Mammalian mitochondrial IAP binding proteins. Biochem Biophys Res Commun 2003;304: 499–504.
4. Du C, Fang M, Li Y, Li L, Wang X. Smac, a mitochondrial protein that promotes cytochrome c–dependent caspase activation by eliminating IAP inhibition. Cell 2000;102:33-39.
5. J.D. Lochrie, K. Phillips, E. Tonner, et al., Insulin-like growth factor binding protein (IGFBP)-5 is upregulated during both differentiation and apoptosis in primary cultures of mouse mammary epithelial cells, J. Cell. Physiol. (2006);207:471-479.