PublisherInfo		
PublisherName	:	BioMed Central
PublisherLocation	:	London
PublisherImprintName	:	BioMed Central

Gene therapy that results in apoptosis of synovial fibroblasts

ArticleInfo		
ArticleID	:	177
ArticleDOI	:	10.1186/ar-2000-66819
ArticleCitationID	:	66819
ArticleSequenceNumber	:	134
ArticleCategory	:	Paper Report
ArticleFirstPage	:	1
ArticleLastPage	:	3
ArticleHistory	:	RegistrationDate : 2000–6–14 OnlineDate : 2000–6–14
ArticleCopyright	:	Current Science Ltd2000
ArticleGrants	:	
ArticleContext	:	130753311

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Keywords

Arthritis, gene Therapy, NF-?B, XIAP

Context

A prominent feature of the rheumatoid synovium is the dysregulated hyperplasia of SFs due to a postulated imbalance between growth and apoptosis signals. Stimulation of SFs from RA patients with TNF-a results in enzyme secretion, which may contribute to articular destruction. Induction of apoptosis of this fibroblast population may represent a potential therapeutic approach in RA. Stimulation of cells by TNF-a can generate two signals: one initiates apoptosis, whereas the second leads to activation of NF-?B (which in turn produces inhibitors of apoptosis [IAPs] and promotes the production of pro-inflammatory factors). To induce an apoptotic response in RASFs to TNF-a through adenoviral expression of a truncated stable form of I?Ba and expression of an antisense fragment to XIAP.

Significant findings

AdCMVI?B-DN expressed a truncated form of I?Ba by western blot, that efficiently inhibited TNF-a induced NF-?B nuclear translocation in RASFs. RASFs were resistant to TNFa-mediated apoptosis; however, this resistance was lost upon transduction with AdCMVI?B-DN, associated with caspase 3 activation. In the *in vivo*SCID mouse model of RASF hyperplasia, extensive apoptosis was only observed in joints that received AdCMVI?B-DN in combination with systemic TNF-a. The degree of bone erosion was similar for all treatment groups. XIAP was shown to be induced in RASFs by TNF-a in a dose-dependent manner through an NF-?B dependent mechanism, since it could be blocked by infection of cells with AdCMVI?B-DN. The contribution of XIAP to the resistance of RASFs to TNF-a apoptosis was determined by infecting RASFs with AdCMVXIAP-AS or the control GFP construct and then exposing the cells to TNF-a (10 ng/ml) for 12 h. RASFs infected with AdCMVXIAP-AS displayed up to 80% apoptosis after TNF-a treatment compared to control cells.

Comments

This interesting study extends the work of Miagov *et al*, through the use of human rheumatoid arthritis synovial fibroblasts (RASFs) *in vitro* and in an *in vivo* SCID mouse model of synovial hyperplasia. Blockade of NF-?B with a stable mutant I?Ba deviates the response of RASFs to tumour necrosis factor (TNF)-a from non-apoptotic to an apoptotic pathway both *in vitro* and *in vivo*. These data further support the therapeutic potential of targeting NF-?B. In addition the same effect is achieved *in vitro* through blockade of the downstream X-linked inhibitor of apoptosis (XIAP). XIAP may represent a more selective apoptosis-inducing target compared with the numerous transcriptional targets of NF-?B; however, its precise functions require further characterisation. Whilst fragments of XIAP are known to inhibit caspase activity, a recent study has shown XIAP can induce NF-?B activation in a regulatory loop. Further *in vitro* studies on normal synoviocytes and *in vivo*studies in experimental models of arthritis will clarify the therapeutic potential of targeting XIAP expression/function.

Methods

Primary synovial cell lines were established from tissue obtained from patients undergoing total knee replacement for RA. Adenoviral constructs encoding a mutated I?Ba (AdCMVI?B-DN), an antisense XIAP fragment from -34 to +80 (AdCMVXIAP-AS), green fluorescent protein (GFP) (AdCMVGFP) and LacZ (AdCMVLacZ) were used in this study. The effect of transduction of RASF with AdCMVI?B-DN or AdCMVGFP *in vitro*was assessed as follows: nuclear translocation of NF-?B in response to TNF-a was assessed by gel shift analysis; TNF-a-induced caspase 3 activation was determined by western blot; apoptosis in the absence or presence of TNF-a was measured by Hoechst 33258 staining. Injection of RASF intra-articularly in both knees of SCID mice manifests fibroblast hyperplasia after 4 weeks. These fibroblasts were then transduced by inta-articular injection of AdCMVI?B-DN or AdCMVLacZ with or without TNF-a (10 ?g/kg) given systemically 2 days later. Hyperplastic growth was determined histologically by TUNEL staining of tissue obtained 24 h later. The expression of XIAP in RASFs in response to TNF-a was assessed by northern blot and RT-PCR. The effect of blocking XIAP expression, by transduction of RASF with AdCMVXIAP-AS, on TNF-a induced apoptosis was determined by Hoechst staining.

References

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