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MODULATION OF CELLULAR FUNCTIONS IN CULTURED GRAVES' RETROOCULAR FIBROBLASTS USING ANTISENSE OLIGO-NUCLEOTIDES TARGETING THE C-MYC PROTO-ONCOGENE.

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Alterations of the connective tissue compartment within the orbit play a central role in the evolution of Graves' ophthalmopathy (GO). The enhanced proliferative and metabolic activities of retroocular fibroblasts in GO are thought to result, at least in part, from paracrine and autocrine signals delivered both by infiltrating T cells and residential cells. Although inhibition of effector cell functions may have therapeutic implications, the signal transduction pathways involved in fibroblast activation have not yet been explored. Using a panel of oligomers complimentary to the translation initiation region of the human proto-oncogene c-myc, we have studied the effects of inhibition of c-myc expression. Antisense 16-mer phosphorothioate oligodeoxynucleotides (S-ODN) concentrations of 1-12 μM markedly reduced the proliferative capacity of cultured Graves' retroocular fibroblasts compared to cells treated with sense or randomerized oligomers of equal length and GC content ($p<0.001$). Inhibition of cell proliferation, as determined by a non-radioactive cell proliferation assay, was 78% - 96% at 24 h with significant inhibition of 58% - 87% maintained through 96 h of cell culture. No cell cytotoxicity or changes in cell viability were observed at these concentrations. In addition, stimulation of cell proliferation by IL-1α (10 U/ml) and PDGF (1 ng/ml) of retroocular fibroblasts was also markedly inhibited by these anti-c-myc S-ODN (91% and 94 % at 24 h, 66% and 74% at 96 h, respectively). Further, c-myc antisense oligomers were capable of diminishing glycosaminoglycan (GAG) synthesis by Graves' retroocular fibroblasts both at baseline ($p<0.01$) and following stimulation with IL-1α (10 U/ml; $p<0.001$) and TGFβ (10 ng/ml; $p<0.001$). In conclusion, activation of the c-myc gene may play an important regulatary role in the proliferation and GAG synthesis of Graves' retroocular fibroblasts. The inhibitory effects of c-myc antisense S-ODN on the proliferative and metabolic activity of these cells provides a strong rationale to further study the effects of antisense strategies targeting c-myc and possibly other proto-oncogenes. Loss of function analysis using antisense oligonucleotides for the suppression of oncogenes or overexpressed growth factors may be a valuable tool for studying gene functions of potential relevance to the pathogenesis of GO.

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