To demonstrate the antisense inhibition, we chose phytoene desaturase (pds) as a model gene which is a key-enzyme of the carotenoid biosynthesis in Triticum aestivum and Nicotiana benthamiana. Selection of the antisense target sites was made by a multistep optimization process which raises the targeting efficiency significantly. By means of quantitative RT-PCR method, we demonstrated sequence-specific knock-down of pds mRNA level. We followed the phenotypical changes of the plants by chlorophyll fluorometry and carotenoid content measurement, thus significant loss PDS function was demonstrated. at a significant level. Our experiences open the way for applying the antisense oligonucleotide technique for elucidation of real genetic problems.

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## Epigenetic changes in tumor-associated myofibroblast

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The tumor microenvironment is an important factor in cancer development and progression.

In the stroma of various epithelial tumors the predominant cell types are myofibroblasts. These spindle-shaped cells were originally described in skin wounds where they facilitate wound healing. Myofibroblasts are differenciated fibroblasts expressing specific markers like alpha smooth muscle actin, vimentin and secreting several extracellular matrix proteins (Desmoulière et al. 2004). Compared to normal tissue, the number of myofibroblasts is increased in the tumor stroma and the shape and distribution of the cells are altered as well. The observed morphological changes are believed to be partially due to epigenetic effects, which cause an altered gene expression profile without influencing the DNA sequence. The epigenetic changes occuring in tumor- associated myofibroblasts are poorly understood (Jiang et al. 2008).

In order to compare epigenetic characteristics and gene expression pattern of tumor-associated myofibroblasts with that of normal myofibroblasts in molecular level, we used primary myofibroblast cultures obtained from the gastrointestinal tract. Cells isolated from tumor stroma or from healthy tissues near the tumor margins were provided by Dr. Peter Hegyi (Dept. of Internal Medicine, Faculty of Medicine, University of Szeged). We studied epigenetic changes such as histone H3 and H4 acetylation and methylation in tumor-associated myofibroblasts by immunocytochemistry. The results indicated lower levels of histone H4 acetylated on lysine 8, 12, 16 and H3 dimethylated on lysine 9 in tumor-associated myofibroblast compared to normal cells. Semi quantitative determination of the level of particular histone modifications by immunoblots using modified histone specific antibodies supported and validated the observed epigenetic alterations. The expression profile of subunits of histone acetyltransferase (HAT) complexes were determined by quantitative RT-PCR. The analysis indicated that the mRNA levels corresponding to the *ada2a*, *ada3* and *gcn5* genes, which code subunits of several HAT complexes, such as SAGA and ATAC, were lower in tumor-associated samples, then in their wild type counterparts.

The role of myofibroblasts in cancer metastasis is also suspected (De Wever et al. 2008). They can secrete many proteolytic enzymes, which digest extracellular matrix in order to promote cancer cell invasion. Therefore we were interested in studying the expression, secretion and activity of the gelatinase enzymes, matrix metalloproteinase 2 and 9 (MMP-2, 9) in the myofibroblast cultures. Based on quantitative RT-PCR data we performed, we concluded that the expression of MMP-2 was elevated in tumor-associated myofibroblasts, while the messenger of MMP-9 was detectable neither in the tumor-associated nor the control cells. We have also performed a gelatin zymography to detect the activity of MMP-2 and 9. For this protein extracts from tumor associated and normal cells, and as well secreted protein samples obtained from the culture media were loaded onto polyacrylamide gels co-polymerized with gelatin and resolved under nondenaturing conditions. Development of the gels with protein specific stain indicated strong MMP-2 activities in both samples, while the tumor-associated myofibroblasts secreted more MMP-2 enzymes to the extracellular space.

In the forthcoming months we plan to further investigate the differences in the gene expression profile of tumor-associated versus control myofibroblast using microarray. We expect that the results will broaden and refine our understanding on the role of myofibroblasts in tumor formation and invasion.

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