FP-53  LACK OF COLLAGEN XVIII/ENDOSTATIN RESULTS IN EYE ABNORMALITIES

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Col18a1, encoding α1(XVIII) collagen, was targeted by homologous recombination with a PGK-neo inserted into exon 30 of the gene and collagen XVIII null mice were generated. Mice lacking collagen XVIII and its proteolytically derived product endostatin show delayed regression of hyaloid vessels in the vitreous along the surface of the retina after birth, decreased upregulation of VEGF in retinal neuroglial cells and lack of or abnormal outgrowth of retinal vessels. This suggests that collagen XVIII/endostatin is critical for normal blood vessel formation in the eye. All basement membranes in wild type eyes except Descemet's membrane showed immunogold-labeling with antibodies against collagen XVII. Strong labeling at sites where collagen fibrils inserted into the inner limiting membrane in wild type mice and separation between fibrils and the inner limiting membrane of mutant mice indicate that collagen XVIII is important for anchoring vitreal collagen fibrils to the inner limiting membrane. The findings provide an explanation for high myopia, vitreoretinal degeneration and retinal detachment seen in patients with Knobloch syndrome caused by loss of function mutations in collagen XVIII.

FP-54  THE ANTI-TUMOR EFFECT OF ENDOGENOUS ENDOSTATIN PRODUCED BY THE HUMAN CELL LINE

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Background: Endostatin, C-terminal fragment of collagen XVIII, has been reported to be an angiogenic inhibitor and is currently undergoing clinical trials as a new anticancer drug in USA. However, the mechanism that endostatin inhibits angiogenesis is still unknown. Our aim was to elucidate whether endostatin had anti-tumor activity in hepatocellular carcinoma (HCC). Method: We transplanted human HCC cells (JHH-1) into nude mice to develop solid tumors and administered anti-endostatin antibody (CH18B). The antibody was established against synthetic peptides of human endostatin and confirmed to be specific for human molecule. We daily injected CH18B and an irrelevant IgG2a monoclonal antibody (control), weekly measured tumor size and resected tumors after 4 weeks. The expression and localization of human endostatin in the tumors were examined immunohistochemically. The apoptotic and the proliferative index were assessed by TUNEL method and anti-phosphorylated histone H3 monoclonal antibody, respectively. Results: In western blot analysis, we realized that JHH-1 produced the protein detected with CH18B. CH18B-treated mice had significantly larger tumors (CH18B: 533 ± 142 mm3 vs. control: 257 ± 44 mm3, p<0.01 at day 28) and developed more capillaries on the tumor surfaces. Human endostatin localized at basement membranes between the tumor and the stroma. The number of apoptotic cells treated with CH18B was significantly lesser (p<0.01), whereas there was no difference in the HCC cell proliferation. Conclusions: Anti-endostatin antibody inhibited the induction of apoptosis by blocking endogenous endostatin and promotes tumor growth. This is the first report of the anti-tumor effect of endogenous endostatin produced by the human HCC cell line by the novel way.