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Abstract (poster session)

Study of the APC gene function in the mouse APC+/APC1638N model

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Familial adenomatous polyposis (FAP) is an autosomal dominant disease characterized by the presence of many of polyps in the colon is induced by germline mutation in the APC gene. If not removed prophylactically they represent a risk of developing colon cancer with an almost 100% penentrance. One of the possibilities of cancer prevention could be an alternative gene therapy using recombinat bacteria as vectors for delivery of therapeutic APC protein. The first step of this work was cloning of a complete APC gene into the vector for expression in bacterial cells. For this purpose the vector pET24a+ was used and expression was performed in E. coli BL21(DE3) pLysS. After transformation, accuracy of the complete APC gene was tested by sequencing. Expressed APC protein was identified by Western blotting using monoclonal and polyclonal antibodies. Recombinant bacteria were orally applied into transgene mice APC+/APC1638N carrying a mutated APC gene resulting in the production of nonfunctional protein and consequently formation of intestinal tumors. Potential reduction of intestinal tumor formation after application were analyzed. The whole gastrointestinal tract was investigated macroscopically, microscopically and immunohistochemically with the use of polyclonal rabbit antibody against APC protein. Also we analysed cytokines (IL-1b; IL-2; IL-4; IL-5; IL-10; IFN-gamma; TNF-alpha) in mouse serum using Bio-Plex ProTM Assay. All four trangenic mice without therapy developed adenomatous polyps in the gastrointestinal tract. Six transgenic mice treated by oral administration of bacteria expressing the APC gene, developed polyps in 33,3 % of two cases. The remaining four mice 66,7 % were without polyps development and immunohistochemistry confirmed in all parts of the gastrointestinal tract positive APC protein more or less strong intensity. We observed positive effect of this therapy at mouse model. The expression of APC protein by non-pathogenic bacteria may be suitable for clinical use as a potential drug.
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