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KNOCKDOWN OF IGF-1R BY ANTISENSE OLIGODEOXYNUCLEOTIDE AUGUMENTS THE SENSITIVITY OF BLADDER CANCER CELLS TO MMC

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Background and Aim: Transitional cell carcinoma (TCC) of the bladder represents the fifth most prevalent malignancy in Western population, with peak incidence found in males of the 50- to 70-year-old age group. A major problem in the management of bladder cancer is the low sensitivity of a large proportion (approximately 40%) among bladder tumors to chemotherapy and the high risk for recurrence of bladder tumors after transurethral resection. So drug resistance, especially in its multiple type forms, remains a major and difficult problem to resolve in bladder cancer therapy. This phenomenon has often been ascribed to strictly pharmacologic factors, such as the overexpression of multidrug transporters P-glycoprotein, multidrug resistance related protein (MRP), and other variables closely implicated DNA repair and induction/modulation of apoptosis, such as p53 and the Bcl-protein family. Furthermore, it has been recently shown that certain growth factors (IGFs etc) may be involved in the mechanism of drug resistance. Clearly, these findings suggest the design of new strategies that might improve bladder tumor response to chemotherapy.

Results have previously shown that human bladder tumor cell lines may be adapted to grow in the complete absence of serum or any other growth supplement and that this can be explained on the basis of autocrine stimulation. The acquirement of autonomous growth capacity was likely to be an important element in the oncogenesis of bladder tumors. Furthermore, criss-cross experiments showed that supernatants stimulated not only proliferation of the autologous cell line of bladder cancer, but also growth of the other bladder cancer cell lines, suggesting the production of common autocrine factors in bladder tumor cells. Some factors or their receptors involved in autocrine loop mechanism

of bladder tumor cells have been confirmed, such as IL-6, the epidermal growth factor receptor, IFN-beta, transferrins-like substance etc. But certain factors which may play key roles in autocrine mechanisms of bladder cancer cells remain to be definite and their physiological mechanism remain to be fully understood. More are still needed to know about the role of IGF1R signaling in bladder cancers.

The following problems remain to be investigated in bladder cancer cells, about which the present studies are concerned:Is IGFs/IGF-1R signaling pathway involved in autocrine growth of human bladder cancer cells and how does bladder instillation drugs such as MMC affect the autocrine expression of bladder cancer cells? Can targeting against IGF1R gene can significantly enhance drug sensitivity of urinary bladder cancer cells to chemotherapy? ③ What potential intracellular signaling mechanisms are involved in IGF1R blockage? ④ May IGF1R self-stablized antisense ODN serves as a potential therapeutic approach to bladder cancer?

To investigate whether IGF-1R was involved in drug resistance of bladder cancer cells.

Methods: RT-PCR was used to detect the mRNA expression of IGF-I, IGF-II, and IGF-IR in T24 cells and normal urothelial cells. Flow cytometry and MTT tests were used to assess the effect of antisense oligodeoxynucleotide (ODN) on drug sensitivities and apoptosis of T24 cells to mitomycin (MMC). Western blot was used to analyze the effect of ODN on expression of IGF-IR protein.

Results: mRNA of IGF-I, IGF-II, and IGF-IR were strongly expressed in serum-free cultured T24 cell line, whereas normal urothelial cells did not express these factors/receptors or only in trace levels; knockdown of IGF1R by antisense ODN significantly inhibited the growth of bladder cancer cells and enhanced sensitivity and apoptosis of T24 cells to MMC.

Conclusion: These results suggested that blockage of IGF-R signaling might potentially contribute to the treatment of bladder cancer cells which are insensitive to chemotherapy.