form an antiparallel homodimer based on the characteristics of high affinity interaction between the amino (N) and carboxyl (C) termini of the receptor. Recently, it is suggested that AR N-to-C interaction is critical for the ability of this receptor to up-regulate the transcription of androgen-responsive genes, and may be a new target for treatment of prostate cancer. In this study, we investigated the effect of N-terminal (1-34) peptide of AR (ARN34) on androgen-dependent function in prostate cancer cell.

**Material and methods:** We constructed a plasmid, pTriARN34, expressing ARN34 by cytomegalovirus promoter. To measure the *in vivo* interaction of the amino terminal domain and ligand-binding domain of AR, we used the mammalian two-hybrid system. Stable clones of LNCaP cells expressing ARN34 were selected with medium containing of G418.

**Results:** Transfection of pTriARN34 suppressed dihydrotestosterone (DHT)-dependent N-to-C interaction of AR in a dose-dependent manner. On AR-mediated reporter gene assay, the expression of ARN34 suppressed DHT-dependent prostate specific antigen transcription. ARN34 also suppressed AR nuclear translocation induced by DHT. Stable expression of ARN34 suppressed androgen-dependent cell growth of LNCaP cells. Moreover, this inhibitory effect of ARN was also confirmed in hydroxyflutamide-induced mutated AR transactivation and cell growth. Treatment of LNCaP cells with 1 nM DHT drove transition of cells from G1 to S-phase. On the other hand, the ectopic expression ARN34 led to cell cycle arrest by inhibiting the entry into S phase in LNCaP cells.

**Conclusions:** Our results demonstrate that disruption of AR N-to-C interaction caused by ARN34 leads to AR dysfunction and inhibition of AR-mediated prostate cancer cell growth. This approach is thus considered to provide a useful therapeutic opinion for blocking AR-mediated prostate cancer growth.

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## Adenoviral transfer of a natural antisense to survivin mRNA down-regulates survivin expression and promotes apoptosis in breast cancer

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**Background:** Survivin, a member of the inhibitor of apoptosis (IAP) family of proteins, is mostly expressed in malignant cells in adult and recognized as a good target for cancer gene therapy. Previously we demonstrated that induction of a natural antisense of survivin, effector cell protease receptor-1 (EPR-1) down-regulated survivin expression with decrease of cell proliferation, increase of apoptosis, and increase of sensitivity to anticancer agent (Yamamoto et al. European Journal of Cancer, 2002; 38:2316). In this study, we constructed a replication defective adenoviral vector encoding the same antisense sequence to survivin and attempted to enhance the efficacy of previous study. By demonstrating an effect of survivin modulation, we ultimately would like to explore a strategy of gene therapy only toxic to malignant cells expressing survivin.

**Material and methods:** Breast cancer BSMZ cell line was established by one of the authors (Watanabe et al. Cancer Research, 1992; 52:5178). An adenoviral vector encoding antisense RNA to survivin was constructed by homologous recombination of adenovirus type 5-derived pJM17 and shuttle plasmid, pCMV-EPR-1 in HEK 293 cells.

**Results:** We infected the vector to BSMZ cells with multiplicity of infection (MOI) of 0, 1, or 5. Cells were harvested, then transcription and expression of survivin were monitored. Northern blot demonstrated that signals of transduced EPR-1 increased MOI-dependently. Correspondingly, cellular levels of survivin decreased 72-hours after viral infection. In cell cycle analysis, down-regulation of survivin caused increased population in the fraction of apoptotic cells (sub-G1 peak) (MOI=0: 4.88%, MOI=1: 5.05%, MOI=5: 11.54%) with decrease in the S phase population (8.78, 9.94, 10.28%, respectively). Cytotoxic assay revealed that transduction of antisense conferred MOI-dependent sensitivity of docetaxel, a chemotherapeutic agent to BSMZ cells.

**Conclusions:** Current study demonstrated that adenoviral transduction of antisense sequence to survivin mRNA down-regulated survivin expression and increased apoptotic fraction. Moreover, it sensitized cells to docetaxel. Since survivin is expressed primarily in malignant cells, our results suggest possible cancer gene therapy with no adverse effect on normal tissues which do not express survivin. Enhancement of chemosensitivity by modulation of survivin may also have a roll for further development of therapies to drug-refractory malignant tumors.

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## Expression of functional CXCR4 on colorectal human cancer.

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**Background:** The chemokines are small proteins known to direct hematopoietic cells to home-specific anatomical sites. The chemokine receptor for SDF1-alfa chemokine, CXCR4, has been implicated in cancer metastasis. Emerging data suggest that it has a key role in determining the metastatic destination of tumor cells as demonstrated in breast, melanoma, ovary, and lung cancer. Since the expression of the CXCR4 receptor may be of prognostic value we studied the expression of CXCR4 on human colorectal cancer.

**Methods:** CXCR4 expression was examined by immunohistochemical staining on paraffin-embedded sections of normal colorectal mucosa (14), iperplastic polypi (6), dysplastic polypi (27), 16 primary carcinomas and 5 hepatic metastasis. CXCR4 expression was also studied by flow cytometry on Caco2, GEO, SW480, SW48, Lovo and SW620 human colorectal cancer cell lines. The effect of SDF1-alfa and liver-derived proteins on migration of cell lines was measured using transwell inserts (8 1/4 m diameter) and 24-well plates. The inhibitory effect of anti-CXCR4 antibody (10 1/4 g/ml) on migration was also studied.

**Results:** CXCR4 staining resulted weakly positive in 6 and strongly positive in 1 (infiltrated by melanoma) out of 14 samples of normal mucosa, clearly positive in 19 out 27 dysplastic lesions with higher staining intensity for moderate/poorly differentiated lesions (13/17, moderate/poorly vs 6/10, well differentiated), and dramatically positive in 16 out of 16 carcinomas.

SW480, SW48 and SW620 human colon cancer cell lines showed the highest levels of the CXCR4 (60-80% of positive cells), 30-60% for Caco2 cells, 20% Lovo cells and 5-10% GEO cells compared to the 50% of the MDA231 human breast cancer cell line considered to be an ephitelial cell line overexpressing CXCR4 and to the 8% of the HT1080 human fibrosarcoma cell line.

In order to verify the functional status of CXCR4, the ability to migrate versus its natural ligand was assayed on SW480 human colon cancer cells. Preliminary results showed that SW480 migrate in response to SDF1-alfa chemokine relatively to the expression of CXCR4. Furthermore the neutralization of CXCR4 by antibodies inhibits *in vitro* the migratory response to purified SDF1-alfa as well as to liver-derived proteins. Thus the overexpressed CXCR4 is functional.

**Conclusions:** These preliminary results showed CXCR4 overexpression on human colon cancer tissue compared to normal mucosa and benign lesions. Experiments on human colon cancer cell lines suggest a functional activity of CXCR4. Studies on the possible prognostic role of the CXCR4 expression in patients bearing colorectal cancer and its eventual role for targeted therapy are warranted.

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## Therapy of MHC class I+ and class I- HPV16-associated tumours with IL-2, IL-12, and genetically modified tumour vaccines

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**Purpose of the study:** To examine local and systemic effects of IL-2, IL-12 and genetically modified tumour cell-based vaccines directed against HPV16-associated neoplasms in experimental model systems.

**Experimental models:** Moderately immunogenic, MHC class I-negative MK16/1/IIIABC (MK16) cells were previously established by co-transfection of HPV16 E6/E7 and activated Ha-ras DNA into C57 BL/6 murine kidney cells. The MK16 cells formed s.c. tumours in syngeneic mice and metastasized to lungs and lymph nodes (Smahel, Sobotkova, Bubenik et al., Br. J. Cancer 84:374-380, 2001). For comparison, MHC class I-positive, non-metastasizing TC-1 cells, established by co-transfection of C57BL/6 murine lung cells with E6/E7 HPV16 and activated Ha-ras DNA (Lin, Guarnieri, Staveley-O'Carroll et al., Cancer Res. 56:21-26, 1996) were utilized.