

6145 **ALEX1 Suppresses Colony Formation Ability of Human Colorectal Carcinoma Cell Lines and Contributes to a Better Prognostic in Colorectal Cancer**

POSTER

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Introduction: Arm protein lost in epithelial cancers, on chromosome X (ALEX) is a novel subgroup within the Armadillo family. The ALEX/ARMCX gene family consists of six genes including three predicted genes but little is known about the ALEX/ARMCX genes. Both ALEX1 and ALEX2 mRNA is expressed in a variety of adult human tissues, but dramatically reduced or undetectable in several human carcinoma cell lines or human tissues. The role and the expression profile of ALEX1 gene in colorectal tumour are not well examined. Here we evaluated the effects of ALEX1 overexpression on colony formation ability of human colorectal carcinoma cell lines and expression of ALEX1 mRNA in human colorectal tumour in comparison with the normal tissues.

Materials and Methods: Human colon cancer cell lines (HCT116, SW480) and breast cancer cell line (MCF-7) were used in this study. Tumour specimens along with adjacent normal tissues were obtained from 23 patients with primary colorectal cancer and quantitative real-time RT-PCR for samples was performed using the Power SYBR Green PCR Master Mix. Anti-human ALEX1/ARMCX1 polyclonal antibody and anti- β -actin monoclonal antibody were used at a dilution of 1:1,000 and 1:10,000 in Western blot analysis. For bisulfite genomic sequencing, genomic DNA was purified by the phenol chloroform extraction and bisulfite treatment of the genomic DNA was carried out with the EpiTect bisulfite kit. Human ALEX1 gene was amplified by PCR and inserted into the XhoI site of pCAGIPuro plasmid. Plasmid transfections were performed by LipofectAMINE 2000 or LipofectAMINE LTX. Colony formation assay and Soft agar colony formation assay were performed to examine the effect of overexpression of the ALEX1 on cancer cell proliferation.

Results: Overexpression of ALEX1 in colorectal carcinoma cells was capable of impairing colony formation and suppressed the anchorage-dependent and -independent colony formation of human colorectal carcinoma cell lines by the study of stable clones of HCT116 cells expressing ALEX1 protein. Bisulfite genomic sequencing revealed that the promoter region of ALEX1 gene was highly methylated in both HCT116 and SW480 cells in comparison to those in PANC-1 and MCF-7 cells which express endogenous ALEX1 mRNA, indicating the capability of promoter methylation to silence ALEX1 gene in HCT116 and SW480 cells. ALEX1 mRNA was significantly reduced ($P < 0.001$) in 17 cases out of 23 human colorectal tumour tissues than adjacent normal mucosa tissues. Colorectal cancer patients with ALEX1 (Tumour/normal value > 0.0017 ; $n = 12$) showed significantly better prognosis than those without ALEX1 ($n = 11$) ($P = 0.0239$).

Conclusions: Overexpression of ALEX1 suppresses the colony formation activities and is silenced by DNA hypermethylation in colorectal carcinoma cell lines. These findings suggest that overexpression of ALEX1 play a negative role in human colorectal tumorigenesis.

6146 **Preclinical Study of Adoptive Immunotherapy With Natural Killer Cells in Combination With Anti-EGFR Monoclonal Antibodies and Cytokines in Metastatic Colorectal Cancer**

POSTER

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Background: Randomized studies have demonstrated that metastatic colorectal cancer (CRC) patients benefit from anti-EGFR monoclonal antibodies (mAbs) therapy only in the absence of a mutation in the KRAS gene. With the aim to evaluate whether KRAS mutated CRC cells may be susceptible to anti-EGFR-induced ADCC mediated by natural killer (NK) cells, we investigated the capacity of donor-derived or patient-derived NK cells to lyse a panel of CRC cell lines and primary CRC tumour cells.

Materials and Methods: CD56⁺CD3⁻ NK cells purified from peripheral blood mononuclear cells, were incubated overnight with medium alone or stimulated with IL-2 (100U/ml) or IL-15 (20U/ml) and tested against 51Cr-labeled CRC cell lines or primary CRC tumour cells in NK assay or in ADCC assay after pre-incubation with Cetuximab. SW48, KRAS wild-type and HCT-116 KRAS mutated cell lines were chosen as targets for ADCC assay. Primary tumour cells were successful in vitro growth starting from tumour samples obtained after surgery in 10 different CRC patients and analyzed for a series of molecules involved in NK mediated killing included the ligands of activating NK receptors present on effector cells.

Results: All 7 CRC cell lines, including those harbouring KRAS mutation, were susceptible to unstimulated allogeneic NK cells lysis (mean $20\% \pm 4$) and lytic activity is significantly enhanced ($p < 0.05$) by either IL-2 or IL-15 pre-activation (mean $48 \pm 5\%$). By cetuximab pre-coating, SW48 and HCT-116 lysis increased from $38 \pm 4\%$ to $50 \pm 7\%$ ($p < 0.05$) and from $20 \pm 3\%$ to

$36 \pm 5\%$ ($p < 0.05$), respectively. IL-2 or IL-15 pre-activated NK cells significantly increased ($p < 0.05$) lysis of both CRC cell lines. These results refer to an effector:target ratio of 25:1 using both donor- and patients-derived NK cells. Preliminary results obtained in two patients demonstrated that autologous NK cells lysed primary tumour cells in ADCC assay and that the lysis was strongly increased by pre-activation of NK cells with IL-2 or IL-15. **Conclusions:** NK cells from donors or CRC patients are able to lyse all tested CRC lines. IL-2 and IL-15 activation significantly improve NK cytotoxicity which is further enhanced by cetuximab, independently from KRAS gene mutational status. These findings, undergoing confirmation with additional studies, may support a pilot study of NK cells therapy in metastatic CRC.

6147 **A Novel Approach to the Emerging and Frequent Problem of Sustained Long-term Oxaliplatin-induced Neurotoxicity**

POSTER

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Background: Oxaliplatin-induced neuropathy represents a serious limitation of the treatment of colorectal cancer, however the long-term neurological sequelae have not been adequately described. In addition, there remains no objective assessment to predict which patients are most at-risk of severe neurotoxicity. We utilized a novel neurophysiological assessment tool to examine both the development and long-term persistence of oxaliplatin-induced neurotoxicity.

Material and Methods: Clinical grading scales, nerve conduction studies and 905 sensory axonal excitability studies were undertaken in 58 consecutive oxaliplatin-treated patients longitudinally across treatment. A subset of 24 patients was assessed at follow-up of a median 25 months post-treatment.

Results: At long-term follow-up, 76% of patients reported residual neuropathic symptoms and sensory amplitudes remained significantly reduced ($P < 0.005$). Axonal excitability studies revealed cumulative excitability changes in sensory nerves longitudinally across treatment (Refractoriness pre $9 \pm 2\%$; final $-2 \pm 2\%$; $P \leq 0.001$) which had not returned to pre-oxaliplatin levels at follow-up (Refractoriness follow-up $6 \pm 4\%$ $P < 0.05$), suggesting persisting abnormalities in nerve function. Importantly, excitability abnormalities preceded sensory amplitude reduction ($P \leq 0.001$) and the development of neuropathy ($P < 0.01$) and were able to predict clinical outcome at final oxaliplatin treatment in 80% of patients ($P < 0.05$). Crucially, at long-term follow-up, the extent of excitability abnormalities during treatment was significantly correlated with clinical outcomes at follow-up (Correlation coefficient = -0.779 ; $P = 0.003$), suggesting that they represent early markers of long-term neurological sequelae.

Conclusions: Objective and subjective neurological deficits persisted in oxaliplatin-treated patients at follow-up, suggesting that persistent sensory neuropathy is a long-term outcome of oxaliplatin treatment. Importantly, axonal excitability studies obtained during treatment provide early identification of patients at-risk of severe, long-lasting neurotoxicity prior to the development of neuropathy, providing a significant advantage over conventional neurophysiological measures.

6148 **Health Related Quality of Life Analysis of Stage III Colorectal Cancer Patients Receiving Different Adjuvant Treatments**

POSTER

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Background: The objectives of this study were to evaluate the health-related quality of life (HRQOL) and to compare direct cost of stage III colorectal cancer patients receiving either capecitabine (Xeloda®)-based or 5-FU/LV-based adjuvant treatments from a single payer perspective.

Materials: An observational and prospective follow-up study, in conjunction with 10 hospitals in Taiwan, was conducted from July, 2008 to December 2010. A total of 256 patients with stage III colorectal cancer were invited to complete questionnaires during the study period: at the time of inclusion in the study (Q0), at 3 months after the initial adjuvant treatment (Q3), at 1 month after patient had finished the adjuvant treatment (Q7) using the European Organization for Research and Treatment of Cancer (EORTC) QLQ-C30 and QLQCR-38. Direct cost data were obtained from cost questionnaire and National Health Insurance claim files. A total of 200