6145 POSTER

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

A. Takeda[1]. [1]*International University of Health and Welfare Hospital, Surgery, Tochigi, Japan*

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 polyclonel antibody and anti-b-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 hypermetilation in colorectal carcinoma cell lines. These findings suggest that overexpression of ALEX1 play a negative role in human colorectal tumorigenesis.

6146 POSTER

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

S. Brugnatelli[1], D. Montagna[1], I. Turin[1], M. Manzoni[1], L. Castagna[2], M. Maestri[1], I. Grecchi[1], A. Santoro[2], P. Pedrazzoli[1], E. Todisco[2]. [1]*Fondazione IRCCS Policlinico S. Matteo, University of Pavia, Pavia, Italy;* [2]*Humanitas Cancer Center, Hematology-Oncology, Rozzano (MI), Italy*

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 tumour cells in NK assay or in ADCC assay after pre-incubation with Cetuximab. SW48, KRAS wilde-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 POSTER

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

S.B. Park[1], C.S.Y. Lin[2], A.V. Krishnan[2], D. Goldstein[3], M.L. Friedlander[3], M.C. Kiernan[1]. [1]*University of New South Wales, Neuroscience Research Australia, Sydney NSW, Australia;* [2]*University of New South Wales, School of Medical Sciences, Sydney NSW, Australia;* [3]*Prince of Wales Hospital, Department of Medical Oncology, Sydney NSW, Australia*

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 ± 2%; $P \leqslant 0.001$) 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 ($P \leqslant 0.001$) 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 POSTER

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

H.H. Chen[1], W.T.L. Chen[2], H.H.C. Lee[3], J. Lin[4], M.C. Yang[5], E. Lin[6]. [1]*Chang Guang Memorial Hospital Kaohsiung Medical Center, Colorectal Surgery, Kaohsiung, Taiwan;* [2]*China Medical University Hospital, Colorectal Surgery, Taichung, Taiwan;* [3]*Cathay General Hospital, Colorectal Surgery, Taipei, Taiwan;* [4]*Taipei Veterans General Hospital, Colorectal Surgery, Taipei, Taiwan;* [5]*National Taiwan University, Institute of Health Policy and Management, Taipei, Taiwan;* [6]*Roche Products Ltd., Pharma, Taipei, Taiwan*

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

patients (78%) responded. After propensity score matching, a total of 162 patients were involved in this study.
Results: Patients' gender, age, location of tumour, marital status, education, work and number of comorbidity were not significantly different between two treatments. In capecitabine (Xeloda®)-based treatment, Physical, Role, Emotional, Social, Global Health status, Future perspective Functioning, Fatigue, Diarrhea, Defecation problems and Weight loss Symptoms improved from Q0 to Q3 and Q0 to Q7. In 5-FU/LV-based treatment, Physical, Role, Global health status Functioning, Gastrointestinal tract and Weight loss Symptoms also improved. Total cost of capecitabine (Xeloda®)-based and 5-FU/LV-based treatment were € 690 (NT$27,706) and €1,512 (NT$60,691), respectively.
Conclusions: To the authors' knowledge, the current study is the first to examine trends over time regarding the effects of adjuvant treatment on HRQOL and direct cost of colorectal cancer patients. The results indicate that adjuvant chemotherapy for colorectal cancers has no negative impact on the HRQOL. Between the two adjuvant chemotherapies in the study, capecitabine (Xeloda®)-based treatment perform better in HRQOL improvement and has less direct cost.

6149 POSTER

Preliminary Results of a Phase 2−3 Clinical Study With the Immunomodulator MGN1703 in Patients With Advanced Colorectal Carcinoma (IMPACT Study)

M. Schmidt[1], H.J. Schmoll[2], F. Ghiringhelli[3], F. Mayer[4], H. Kröning[5], R. Ziebermayr[6], M. Schroff[1], E. Weith[1], M. Tschaika[1], B. Wittig[7]. *[1]MOLOGEN AG, Berlin, Germany; [2]Martin-Luther-University Halle-Wittenberg, University Clinic and Policlinic for Internal Medicine IV, Halle, Germany; [3]Centre Georges François Leclerc, Dijon, France; [4]University of Tuebingen, Medical Clinic, Tübingen, Germany; [5]Schwerpunktpraxis für Hämatologie und Onkologie Magdeburg, Magdeburg, Germany; [6]Elisabethinen Linz, 1. Interne Hämatologie mit Stammzeltransplantation und medizinischer Onkologie, Linz, Austria; [7]Freie Universität Berlin, Foundation Institute Molecular Biology and Bioinformatics, Berlin, Germany*

Background: MGN1703 is a synthetic DNA-based immunomodulator and acts as an agonist of toll-like receptor 9 (TLR9). Based on the promising data from currently finished phase 1 study in patients with metastatic solid tumours including those with CRC, a phase 2−3 study was initiated in patients with advanced CRC. The objective of the study is to assess safety and efficacy of the MGN1703 treatment in comparison to placebo.
Methods: This randomized double-blinded placebo-controlled phase 2−3 clinical study (IMPACT study; MGN1703-C02; EudraCT number: 2009–017432−40; Sponsor: Mologen AG) is conducted in patients with advanced CRC showing disease control after first-line therapy with standard chemotherapy regimen. The study is conducted in Germany, Austria, France, United Kingdom, Czech Republic and Russia, and 129 patients will be recruited into the study. The patients are subcutaneously treated twice a week either with 60 mg MGN1703 or with placebo (using ratio of 2:1). The efficacy and safety of the study treatment will be evaluated based on extensive immunological tests, radiological assessment, safety laboratory results and assessments of the quality of life. The study treatment will be continued until tumour progression, intolerable toxicity, exclusion criteria, withdrawal of consent or death.
Results: Thirty-two adverse events have currently been reported. Out of those, 26 (81.2%) were assessed as not drug-related by the investigator: The remaining were mild night sweat (not assessable), mild fever (at three occasions, possible related), mild injection site itching (probable related) and mild arthralgia (certain related) in one patient each. One not drug-related SAE − ileus − was reported. Only in single patients local reactions such as mild redness and swelling at injection site were reported. No laboratory or clinical signs of autoimmunity or dose-limiting toxicities were reported, so far.
Conclusions: The preliminary safety results of this ongoing clinical study in patients with advanced CRC show that treatment with MGN1703 at the dosage of 60 mg is well tolerated and safe. Reported adverse events were not accompanied by any signs of autoimmunity.

6150 POSTER

Survival Outcomes With Use of Bevacizumab Beyond Progression (BBP) in Metastatic Colorectal Cancer (MCRC) Patients (Pts)

T. Cartwright[1], E. Yu[2], Y. Yim[2], H. Hartnett[3], H. Chung[3], M. Halm[3], M. Forsyth[3]. *[1]Ocala Oncology, Ocala Oncology, Ocala, USA; [2]Genentech, Health Outcomes, South San Francisco, USA; [3]U.S. Oncology, Healthcare Informatics, Houston TX, USA*

Background: Bevacizumab (BV) prolongs overall survival (OS) when added to chemotherapy (CT) in 1st line (1L) and 2nd line (2L) treatment (Tx) of MCRC. Findings from the observational cohort studies BRiTE and ARIES suggest that continued use of BV beyond the first progression improves survival for MCRC patients. A retrospective analysis was conducted to evaluate the association between survival outcomes and the use of BBP in MCRC Pts treated in the community setting.
Methods: Data was derived from the US Oncology's iKnowMed electronic medical record system. MCRC Pts who received 2L Tx after receiving 1L CT with BV between 7/1/2006 and 6/30/2009 were identified. Date of progressive disease (PD) was defined as initiation of 2L Tx. Pts were followed until death or loss to follow up, whichever came first. Pts were divided into 2 cohorts: Pts who continued BV post-PD in 2L (BBP) and Pts who received post-PD treatment in 2L without BV (No BBP). Clinical outcomes were measured by OS, defined as time from initiation of 1L Tx to death and survival beyond progression (SBP), defined as time from initiation of 2L Tx to death. Baseline characteristics were compared between groups using chi-square analysis for categorical variables and t-tests for continuous variables. OS and SBP were estimated using Kaplan–Meier method. Cox proportional hazards model was used to assess effects of Pt and Tx characteristics on OS and SBP, adjusting for age at 2L start, time to 1st PD, use of anti-EGFR therapies post PD, gender, ECOG performance status at 2L start, time between end of 1L and start of 2L, exposure to three active CT agents (5-FU, oxaliplatin and irinotecan), and primary tumour site.
Results: 641 Pts met criteria for No BBP (n = 368) and BBP (n = 273). Pt and Tx characteristics between groups were similar except age at 2L start (median 62 yrs vs 60 yrs for No BBP vs. BBP), practice region, use of anti-EGFR therapies post PD, and 2L CT. Median OS and SBP were longer in the BBP cohort (OS 28.2 mo; SBP 15.4 mo) compared to the No BBP cohort (OS 21.0 mo; SBP 8.8 mo). BBP was associated with longer OS (HR = 0.64; 95% CI 0.50–0.81), and longer SBP HR = 0.59; 95% CI 0.46–0.75, after adjusting for covariates in the Cox model.
Conclusion: In MCRC patients treated in the community setting, the use of BBP appears to be significantly associated with prolonged OS and SBP. These results are consistent with previous results from large observational cohort studies.

6151 POSTER

Follow up After Hepatectomy for Colorectal Liver Metastases − a Systematic Review and Meta-analysis

R. Jones[1], D. Dunne[1], S. Fenwick[1], G. Poston[1], P. Malik[1], P. Ghaneh[1]. *[1]University Hospital Aintree NHS Foundation Trust, Department of Hepatobiliary Surgery, Liverpool, United Kingdom*

Background: Follow up of patients after liver resection offers two potential benefits; clinicians can assess the efficacy of treatment, and recurrent disease can be detected and treated. The majority of recurrence occurs within the first two years of resection, and many centres concentrate follow up in this period.
Objective: To review the evidence surrounding follow up after liver resection for metastatic colorectal disease and define an evidence base for intensive early follow up.
Methods: A systematic review using databases, trial registers and conference proceedings. We included any studies that described potentially curative primary resection of colorectal liver metastases that included a defined follow-up protocol between Jan 2003 − May 2010. Studies were divided into intensive early follow up and standard follow up based on pattern of review.
Results: 335 studies were identified, of which 35 met the inclusion criteria, involving 7330 patients. Intensive early follow up showed median survival of 39.8 months (95% CI 34.3–45.3), with 1-,3- and 5-year survival of 91.5% (95% CI 83.4–99.6), 57.6% (95% CI 50.7–64.5) and 41.9% (95% CI 34.4–49.4). Routine follow up showed median survival of 40.2 months (95% CI 33.4–47.0), with 1-, 3- and 5-year overall survival of 86.7% (95% CI 78.3–95.1), 52.8% (95% CI 46.9–58.7) and 38.4% (95% CI 32.56–44.3) respectively. Only 5 studies directly assessed the most appropriate method of follow-up after resection, and adopted a combination of radiological and haematological assessment. Most recurrence was detected within 3 years of resection. One study specifically assessing the impact of intensive (3-monthly) CT-based follow-up found it was associated with better overall survival than palliative treatment.
Conclusions: Evidence surrounding follow up after liver resection is poor. Meta-analysis failed to identify an advantage to intense early follow-up.

6152 POSTER

Trends in Survival and Chemotherapy (CTx) Usage in Elderly Patients With Metastatic Colorectal Cancer (mCRC)

V. Shankaran[1], S. Beck[2], D. Blough[3], L. Koepl[2], Y. Yim[4], E. Yu[4], S. Ramsey[2]. *[1]University of Washington, Medical Oncology, Seattle WA, USA; [2]Fred Hutchinson Cancer Research Center, Cancer Prevention, Seattle WA, USA; [3]University of Washington, School of Pharmacy, Seattle WA, USA; [4]Genentech, Health Outcomes, San Francisco CA, USA*

Background: Several drugs [including bevacizumab (Bv), oxaliplatin (Ox), and irinotecan (Iri)] have been approved since 2002 for the treatment of