

microwells were prepared by placing a cover slip (22mm X 22mm) on a 35mm culture dish. Molten agar (3%) was poured on the top of the cover slip enough to cover it (500 μ l approx). The ellipsoidal microwell in PDMS (polydimethylsiloxane) was then placed on the top of the cover slip containing the molten agar and allowed it to solidify. The culture conditions were carried at 37°C, 5% CO₂ and humidified hypoxic conditions (1% O₂). The culture medium was replaced every 48-72 hrs with nominal disturbances to the microwells. Cultures were maintained for 3 weeks and imaged on day 7, 14, 21 with bright field microscope and analyzed the images using (Olympus IX71). **Result:** To correlate cluster formation with survival, samples were obtained from 31 patients with lung cancer. These patients were enrolled, when they were presented with progressive disease since their last treatment regimen but before commencing a new treatment regimen. Blood was collected before and on 9th, 12th week after the treatment. It is interesting to note that tight cluster formation correlated with patient survival. The Kaplan-Meier survival analysis showed that cluster formation in patients who have undergone neoadjuvant therapy correlated with shorter overall survival. Our culture system enabled us to successfully expand the CTCs without any prior enrichment. The method requires 2.5 ml of blood per 35-mm dish to expand CTCs. Microwells were easy to establish and replicate with minimal set-up in a laboratory. **Conclusion:** To the best of our knowledge, this study is one of the few, which suggest the possible role of cluster formation in patient survival in Lung cancer patients. **Keywords:** NSCLC, CTC

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CXCR4 Overexpression is Associated with Poor Survival Outcome After Recurrence in Early Stage Non-Small Cell Lung Cancer Patients



A. Fung,¹ K. Kopciuk,² M. Dean,³ A. D'Silva,¹ S. Otsuka,¹ A. Klimowicz,¹ D. Hao,⁴ D. Morris,⁵ G. Bebb⁶ ¹University of Calgary, Calgary, AB/CA, ²Mathematics and Statistics, University of Calgary, Calgary, AB/CA, ³Medical Oncology, University of Calgary, Calgary, AB/CA, ⁴University of Calgary and Tom Baker Cancer Centre, Calgary, AB/CA, ⁵Medical Oncology, Tom Baker Cancer Centre, Calgary, AB/CA, ⁶Oncology, Tbcc Translational Labs, University of Calgary, Calgary, AB/CA

Background: Overexpression of CXCR4 is associated with poor outcomes for patients with advanced non-small cell lung cancer (NSCLC). Studies suggest a gender specific difference in outcomes of stage IV NSCLC patients, with shorter survival in females with high expression of CXCR4. The current study evaluates the association between CXCR4 expression and gender, time to recurrence, and survival in early stage NSCLC patients. **Method:** Patient characteristics, clinical variables and outcome data were obtained from the Glans-Look Lung Cancer database for stage I-III NSCLC patients diagnosed between 2003-2006 at the Tom Baker Cancer Centre. Tissue microarrays were created from surgical or biopsy specimens, and CXCR4 expression was evaluated using quantitative fluorescent immunohistochemistry. CXCR4 expression and outcome data were analyzed using a Cox proportional hazards and multi-state model. **Result:** 230 patients with stage I-III NSCLC were identified, and 181 patients had corresponding tissue for CXCR4 analysis. Early stage NSCLC patients with CXCR4 overexpression had worse overall survival compared to those with low CXCR4 expression (p<0.05). No gender specific difference was observed. Time to recurrence did not correlate with CXCR4 expression, and there was no association with the site of recurrence (local versus distant). However, high CXCR4 expression was associated with increased risk of death after recurrence (p<0.05). **Conclusion:** Early stage lung cancer patients with high CXCR4 expression have worse survival outcomes, particularly after recurrence of disease. The role of CXCR4 as a prognostic marker in NSCLC patients who have recurred should be further elucidated. **Keywords:** CXCR4, recurrence, survival

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IL-1 β as a New Early Predictive Biomarker for Non-Small Cell Lung Cancers Outcome



S. Missiroli, N. Tamburini, M. Perrone, P. Maniscalco, R. Gafà, G. Lanza, P. Pinton, G. Cavalleco, C. Giorgi University of Ferrara, Ferrara/IT

Background: Lung cancer is the first cause of cancer mortality worldwide. Patients diagnosed with early stage disease (stage I) have been considered to have a reasonably favourable prognosis. Unfortunately, up to 40% early stage cases of all non-small cell lung cancers (NSCLCs) have a poor prognosis. Recent studies have suggested that combined modality treatment of NSCLC may improve survival in selected patients. One of the most critical questions in choosing the appropriate use of combined therapy is determining which patients might benefit from the more aggressive treatment. Recent data have enhanced the idea that inflammation is a critical component of tumour progression. It is now becoming clear that the tumour microenvironment is largely orchestrated by inflammatory cells and it is an indispensable participant in the carcinogenesis. In particular, IL-1b is a pleiotropic pro-inflammatory cytokine and its up-regulation is closely associated with various cancers. Chronic inflammation (sustained by overactivation of IL-1b system) is a crucial event in carcinogenesis and tumor progression and there is evidence that plasma IL-1b level is higher in patients with advanced cancers. Our goal is to determine whether IL-1b expression might be associated with NSCLCs patients' clinical outcome. **Method:** Patients diagnosed with lung cancer stage I and treated with potentially curative resection during 2012 were recruited for this study. Tissue samples (tumor masses including peritumoral stroma and lungs) were collected during surgery, embedded in paraffin and processed for immunohistochemistry (IHC). IHC was performed by ABC-peroxidase. Adjacent sections were incubated for 1 h at room temperature with monoclonal antibody against human IL-1b. **Result:** In our randomized experiment 35 patients with NSCLC whose tumor was resected or biopsied were enrolled during 2012 and followed up for 5 years through 2017. Our preliminary results showed that patients with high levels of IL-1b in the peritumoral area, revealed by IHC, present a poor prognosis and a reduced overall survival. **Conclusion:** This retrospective analysis of patients with stage I NSCLCs who had received standard surgery resulted in statistically significant improvement in overall survival in patients with a low release of IL-1b in the tumor microenvironment. This data define a new role for IL-1 β as a predictive biomarker of NSCLCs tumor behavior that can be used to better profile the use of treatments and improve patients' outcome. **Keywords:** tumor microenvironment, NSCLC, IL-1b

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A Propensity Score Matching Cohort Study on Prognosis of the Diversity of MUC1 Expression in Patients with Lung Adenocarcinoma



C. Gu,¹ Z. Shilei,² L. Zhao,² T. Guo,¹ P. Wang¹ ¹The First Affiliated Hospital of Dalian Medical University, Dalian/CN, ²Department of Thoracic Surgery, The First Affiliated Hospital of Dalian Medical University, Dalian/CN

Background: To probe the expression of MUC1 (Mucin-1) in lung adenocarcinoma tissues, and estimate the relationship between the expression level of MUC1 and prognosis or clinical pathological factors in patients with lung adenocarcinoma simultaneously, so as to establish personal therapeutic strategies and forecast prognosis. **Method:** A retrospective analysis was originally conducted on 182 lung adenocarcinoma patients who underwent surgical resection collected from July 2007 and September 2010 at the First Affiliated Hospital of Dalian