

# Mer and Axl receptor tyrosine kinases are novel therapeutic targets in NSCLC

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Lung cancer is the primary cause of cancer mortality worldwide. Less than 25% of patients treated on current chemotherapy protocols survive 2 years, and new treatment strategies are needed to obtain improved patient outcomes. The identification of new targeted biologic therapies which synergistically interact with standard chemotherapy is one approach to enhance patient survival. Axl and Mer are two related receptor tyrosine kinases and are novel candidates for targeted inhibition since they are abnormally expressed and activated in many human cancers including lung cancer. Studies from other laboratories have demonstrated that expression of Axl correlates with invasive ability of human non-small cell lung cancer (NSCLC) cell lines. Furthermore, in patient samples of NSCLC, Axl overexpression has been statistically associated with metastatic disease. In this study, we used immunohistochemical staining to demonstrate that both Axl and Mer are expressed in the majority of NSCLC patient samples. In human NSCLC cell lines, qRT-PCR and immunoblotting indicate that Axl and Mer, as well as the Gas6 and Protein S ligands which activate them, are co-expressed suggesting that this family of RTKs may constitute an autocrine loop resulting in constitutive activity of these kinases. Knockdown of Axl or Mer expression in the human NSCLC cell lines A549 and H2009 was accomplished via constitutive and inducible lentiviral short hairpin RNA (shRNA) constructs. In vitro assays of cell proliferation and survival indicated that constitutive knockdown of Axl or Mer did not have a significant impact on short-term (48h) growth of NSCLC cells. However, when NSCLC cells were cultured for 8 days to evaluate long-term growth, both Mer and Axl knockdown cells exhibited reduced growth rates relative to control cells. To evaluate the role of Axl and Mer in apoptosis, we performed flow cytometric analysis of cells stained with YO-PRO-1 and propidium iodide. These experiments revealed that constitutive inhibition of Mer, but not Axl, results in increased induction of cell death suggesting that Axl and Mer may be contributing to NSCLC proliferation and survival via distinct mechanisms. Additional in vitro assays demonstrated that inhibition of Mer or Axl significantly increased the sensitivity of NSCLC cells to numerous chemotherapeutic agents. For example, when wild type or non-silencing shRNA control (shControl) cells were treated with carboplatin, an IC50 could not be determined because the cells were robustly resistant to killing with this agent. However, the Axl and Mer knockdown lines demonstrated remarkable sensitivity (IC50 values ~ 9  $\mu$ M and 90  $\mu$ M, respectively) to carboplatin ( $P < 0.05$ ). In other cases, the wild type/shControl lines were sensitive to treatment with chemotherapeutic agents but an increase in sensitivity was observed with Axl or Mer inhibition. Taken together, these results suggest that inhibition of Axl and/or Mer may increase the efficacy of standard chemotherapy and thereby improve patient outcome. While previous studies have suggested a role for Axl in the progression of NSCLC, these studies are the first to describe a role for the related receptor tyrosine kinase Mer in NSCLC or to define a potential synergistic role of Axl or Mer inhibition with commonly used chemotherapy agents. Our findings demonstrate that Mer and Axl are novel biological targets for treatment of NSCLC and possibly a spectrum of other cancers known to aberrantly express Mer and/or Axl.