KRAS Mutation Is Associated with Elevated Myeloblastin Activity in Human Lung Adenocarcinoma

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Abstract. Lung cancer is the leading cause of all cancer deaths worldwide with suboptimal prognosis and treatment options. Therefore this study aimed to identify molecular characteristics with a predictive clinical utility which at the same time might represent novel therapeutic targets for human lung adenocarcinoma. Within this study mutations of v-Ki-RAS2 Kirsten rat sarcoma viral oncogene homolog (KRAS), a gene frequently mutated in lung adenocarcinoma, and their association with enzymatic activities, as assessed by activity-based proteomics, of members of the serine hydrolase (SH) superfamily, a large class of enzymes that have previously been linked to cancer was investigated. The results revealed that the activity of myeloblastin was significantly altered in the lung adenocarcinoma biopsies harboring a KRAS gene mutation. In conclusion myeloblastin is a potential therapeutic target for human lung adenocarcinoma, indicating that the combination of activity-based proteomics with mutational analysis is a valid approach for the discovery of novel biomarkers.

Lung cancer is the major cause of all cancer-related deaths with a worldwide mortality of over one million per year (1). Intense efforts to improve lung cancer outcome have been performed and innovative treatment options, particularly targeted therapies directed against key signaling pathways involved in lung cancer growth and malignant progression have been developed. The most successful example of this approach in the treatment of lung cancer is the development of epidermal growth factor receptor (EGFR) tyrosine inhibitors, which have given substantial benefit to patients with EGFR mutations (2). However, somatic point mutations in KRAS result mainly in amino acid substitutions in exon 2 at codons 12 and 13 and are present in a variety of human malignancies (3, 4). These mutations are among the most frequent genetic alterations in non-small cell lung cancer (NSCLC) and occur in approximately 30% of lung adenocarcinomas, one of the most frequent NSCLC subtypes (5). RAS gene products control a variety of cellular processes, including proliferation, survival and differentiation by regulating the activation of a variety of downstream effector pathways (4). The human RAS genes encode three highly homologous proteins: v-Ha-RAS Harvey rat sarcoma viral oncogene homolog (HRAS), KRAS and neuroblastoma RAS viral oncogene homolog (NRAS) (4, 6, 7). KRAS mutations are correlated with poor patient prognosis in patients suffering from lung cancer (8). In contrast to lung carcinomas harboring EGFR mutations, specific therapies against KRAS mutant lung adenocarcinoma are still lacking since no clinically validated KRAS inhibitors are available (9).

Here, the correlation of KRAS mutations in human lung adenocarcinoma with SH activities, a large class of enzymes that has previously been linked to cancer, was assessed using an emerging methodology termed activity-based proteomics. In this method, chemical structures termed activity-based probes are used to covalently target active enzymes but not their inactive versions (10). Based on SH activities of human lung adenocarcinoma biopsies and matching non-neoplastic tissues as presented in a recent publication, we identified the activities of myeloblastin, a serine protease expressed in macrophages of the lung and pneumocytes (according to www.proteinatlas.org), to be statistically significantly altered in human lung adenocarcinomas harboring KRAS mutations (11).
Materials and Methods

Sample collection. The forty participating individuals had undergone surgery for lung adenocarcinoma at the University Hospital Zurich (UHZ) between 2003 and 2006. Formalin fixed, paraffin embedded (FFPE) as well as snap-frozen lung adenocarcinoma samples were histologically reviewed by one pathologist (A.S.). This study was approved by the local commission of ethics (ref. number StV 29-2009). The characteristics of the patients are summarized in Table I and described in more detail elsewhere (11).

Mutation analysis. For DNA extraction three tissue cylinders (diameter 0.6 mm) were punched from each of the forty paraffin blocks. DNA was extracted according to the QIAGEN EZ1 DNA Tissue protocol for automated purification of DNA from tissue (Qiagen, Hilden, Germany). Mutation detection was performed via a custom service by Sequenom, Inc. using the OncoCarta™ v1.0 panel (Sequenom Inc., San Diego, CA, USA). This panel allows for the parallel analysis of 238 mutations across 19 different oncogenes (12).

Activity-based proteomics and data analysis. Cell extracts derived from the human snap-frozen biopsies were processed prior to mass spectrometric analysis as previously described (11). In brief, the human biopsies were homogenized using a tissue grinder, incubated with the activity-based probe, enriched using streptavidin coated agarose beads (Pierce, Rockford, IL, USA) and on-bead digestion was performed using trypsin (Promega, Fitchburg, WI, USA). Mass spectrometric analysis as well as data analysis was performed as described elsewhere (11).

Statistics. Two-sided, unpaired Student’s t-test was performed for between group comparisons (i.e. mutated versus wild type).

Results

Mutation analysis of the 40 human lung adenocarcinoma specimens identified 4 patients (10%) harbouring a cysteine for glycine substitution at position 12 (G12C) in the KRAS gene (Table I). For technical reasons, the SH activity profiles of “Patient 22” and “Patient 47” needed to be excluded as described elsewhere (11). Out of the four patients harbouring a G12C mutation, myeloblastin activities were not detected in one case. Notably, all four patients harbouring a G12C mutation were previous or current smokers with a smoking history of at least 30 pack years.

By employing the two-sided unpaired t-test a statistically significant (p=0.01) activity difference of the enzyme myeloblastin (UniProtKB/Swiss-Prot ID: P24158) was found between the patients harbouring a G12C mutation (N = 3) in the KRAS gene and the patients harbouring no KRAS mutation (N=34, see Figure 1 and ref. 11).

Discussion

A statistically significant difference in the activity levels of myeloblastin (UniProtKB/Swiss-Prot ID: P24158) was revealed between the biopsies derived from the patients
harbouring a G12C mutation in the \textit{KRAS} gene and the patients harbouring no mutations in the \textit{KRAS} gene. Interestingly, no statistically significant associations between myeloblastin activity levels according to stage, tumor grade, lymph node status or smoking status were detected (11). The down-regulation of myeloblastin has previously been shown to cause growth arrest of leukemic cells (14). Furthermore, myeloblastin has been shown to be involved in the degradation of extracellular matrix (ECM) proteins (15). It has been shown that patients suffering from human lung adenocarcinoma harbouring a G12C mutation are less responsive to cisplatin-based chemotherapy, however, no evidence of an association between the progression of metastatic disease and myeloblastin has so far been presented for human lung adenocarcinoma (16). Importantly, by making use of the publicly accessible gene expression database \texttt{www.genesapiens.org}, no myeloblastin transcript abundance differences were found among normal tissue of the respiratory system compared to lung cancer (13) (Figure 2). This observation indicates that transcript abundances do not necessarily correlate with enzymatic activities and it is important to note that serine protease inhibitors (serpins), a class of inhibitors that comprise approximately 10\% of all plasma proteins, are the major inhibitors of myeloblastin (10, 15). However, the major limitation of this study was the small sample size due to the low frequency of \textit{KRAS} mutations. Roberts and colleagues described in a recent meta-analysis a frequency among current or former smokers and never-smokers of 26\% and 6\%, respectively (5).

From a clinical point of view, it is of special importance that myeloblastin represents a “druggable” target as the activity-based investigation of this enzyme relies on the covalent labelling with an activity-based probe. Furthermore, the observation that myeloblastin transcript levels are not altered in normal tissue of the respiratory system compared to lung cancer indicates that the observed activity differences might not be detectable at the transcript level. Therefore, in our future work we shall aim at validating these results within a larger patient cohort. In addition, based on the identification of myeloblastin protein as a potential biomarker/drug target, it is concluded that the combination of activity-based proteomics with mutational analysis represents a powerful strategy in the search for novel disease biomarkers.

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References


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