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# Editorial:

# HIGHLIGHT REPORT: VALIDATION OF PROGNOSTIC GENES IN LUNG CANCER

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Lung cancer is a leading cause of cancer-related deaths worldwide (Jemal et al., 2010). Therefore, it is not surprising that numerous expression profiling studies have been performed to predict prognosis and response to therapy (Suzuki et al., 2011; Bhattacharjee et al., 2001; Wigle et al., 2002; Tomida et al., 2004; Roepman et al., 2009; Chen et al., 2007; Lohr et al., 2012; Larsen et al., 2007; Stewart et al., 2012; Lu et al., 2006; Guo et al., 2008; Beer et al., 2002; Lee et al., 2008). However, it remains unknown whether the identified prognostic genes add additional prognostic information beyond the traditional clinical parameters (Subramanion and Simon, 2010). The prognostic influence of individual genes has also been critically discussed, since there is almost no overlap between the previously published gene signatures (Botling et al., 2013). To validate the prognostic relevance of individual genes, Botling et al. (2013) recently introduced a novel single institute cohort ('Uppsala cohort') with gene array data from 196 non-small cell lung cancer (NSCLC) patients. They hypothesized that the difficulty to confirm the prognostic role of individual genes may be due to the generally-applied sequential validation strategy, where genes identified in one study are tested for significance in separate subsequent studies (Botling et al., 2013; Lohr et al., 2012, 2013). The size of the individual studies is usually small, which may contribute to the negative results. Therefore, Botling et al. (2013) used an alternative approach - the novel 'Uppsala cohort' was used as a training set to screen for genes significantly associated with prognosis. The 450 probe sets were validated in five publicly-available lung cancer cohorts of 860 additional patients (Botling et al., 2013). However, instead of using a sequential approach, the authors applied a meta-analysis strategy, where as an initial step the association of individual genes was assessed separately in each cohort. The next step involved calculating significance of all studies. This approach is superior to the conventional 'sequential strategy', because it combines the statistical power of several patient cohorts. Fourteen genes, which were found to be significantly associated with survival in the Uppsala cohort, were further confirmed in the meta-analysis (Table 1). One of the identified genes, the adhesion molecule CADM1, was additionally validated using immunostaining (Botling et al., 2013). Validation of prognostic genes seems to be particularly challenging in lung cancer. This observation differs from what has been observed in other tumor entities, such as e.g. breast cancer, where validation of prognostic signatures appears more straightforward (e.g.: Schmidt et al., 2008, 2011, 2012; Hellwig et al., 2010; Brase et al., 2010; Chen et al., 2012). Proliferation, estrogen receptor and immune cell-associated sets of genes have been identified and validated in breast cancer (review: Schmidt et al., 2009; Siggelkow et al., 2012); and redox factors (Cadenas et al., 2010), anti-apoptotic signatures (Petry et al., 2010),

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**Table 1:** Prognostic genes identified and validated in non-small cell lung cancer (from Botling et al., 2013)

| Gene symbol | Gene name and function |
|---|---|
| CADM1 | Cell adhesion molecule 1 |
| PFKP | Phosphofructokinase, platelet |
| SPOCK2 | Sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 2 |
| MECP2 | Methyl CpG binding protein 2 |
| KYNU | Kynureninase (L-kynurenine hydrolase) |
| BZRAP1 | Benzodiazapine receptor (peripheral) associated protein 1 |
| MAP4K4 | Mitogen-activated protein kinase kinase kinase kinase 4 |
| MRPL9 | Mitochondrial ribosomal protein L9 |
| FAM129A | Family with sequence similarity 129, member A |
| AGFG1 | ArfGAP with FG repeats 1 |
| CDCP1 | CUB domain containing protein 1 |
| ERO1L | ERO1-like (*S. cerevisiae*) |
| ARNTL2 | Aryl hydrocarbon receptor nuclear translocator-like 2 |
| EGLN1 | Egl nine homolog 1 (*C. elegans*) |

mechanoactivity (Martin et al., 2012) and senescence (Cadenas et al., 2012) associated genes have also been confirmed. The difficulty in validating prognostic genes in lung cancer may be due to the greater contribution of environmental factors rather than genetic predisposition to the etiology and progression of the disease (Micale et al., 2013; Remmer, 1987; von Stechow et al., 2013). However, the degree to which genetic variability and environmental exposure contribute to cancer risk is still undergoing investigation, and remains highly controversial (Bolt, 2013; Schwender et al., 2012; Hammad et al., 2013; Selinski et al., 2011, 2012a, b, 2013; Golka et al., 2012, 2011, 2009).

In conclusion, the current study of Botling et al. (2013) further illustrates the importance of validating prognostic genes in cancer. The study also demonstrates that the concept of a training set followed by a meta-analysis-based validation in independent cohorts is superior to the conventional approach of sequential cohort by cohort validation.

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