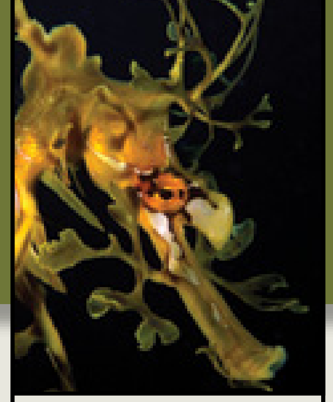

Phosphorylation Signatures Identify Tyrosine Kinases Activated in Lung Cancer

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Introduction

Tyrosine kinase gene amplification and activating mutations occur frequently in certain cancers, for example Her2 is amplified in 30% of breast carcinomas, Flt3 is activated by mutation in roughly 25% of patients with AML, and EGFR is activated by amplification and mutation in 5-15% of NSCLC patients. Finally, the recent success of selective tyrosine kinase inhibitors depends upon the identification of tumors that are driven by activated kinases and are therefore dependent upon the targeted kinase for their survival and clinical benefit. Collectively, these observations emphasize the need to better understand the role of tyrosine kinase activity in human cancer and led us to develop a new method to screen and profile cancer cells for aberrant tyrosine kinase activity. Here we apply a phospho-proteomic approach to identify tyrosine kinases and downstream signaling pathways active in non-small cell lung cancer (NSCLC) cell lines and patients. Over 2000 new sites of tyrosine phosphorylation on over 1,000 different proteins are reported and analyzed to identify signatures of tyrosine kinase activity. We expand the role of deregulated receptor tyrosine kinase signaling in NSCLC to now include PDGFR, Ros, and Alk.

Figure 1. Heterogeneous phospho-tyrosine expression in human lung cancer tissue and cell lines.

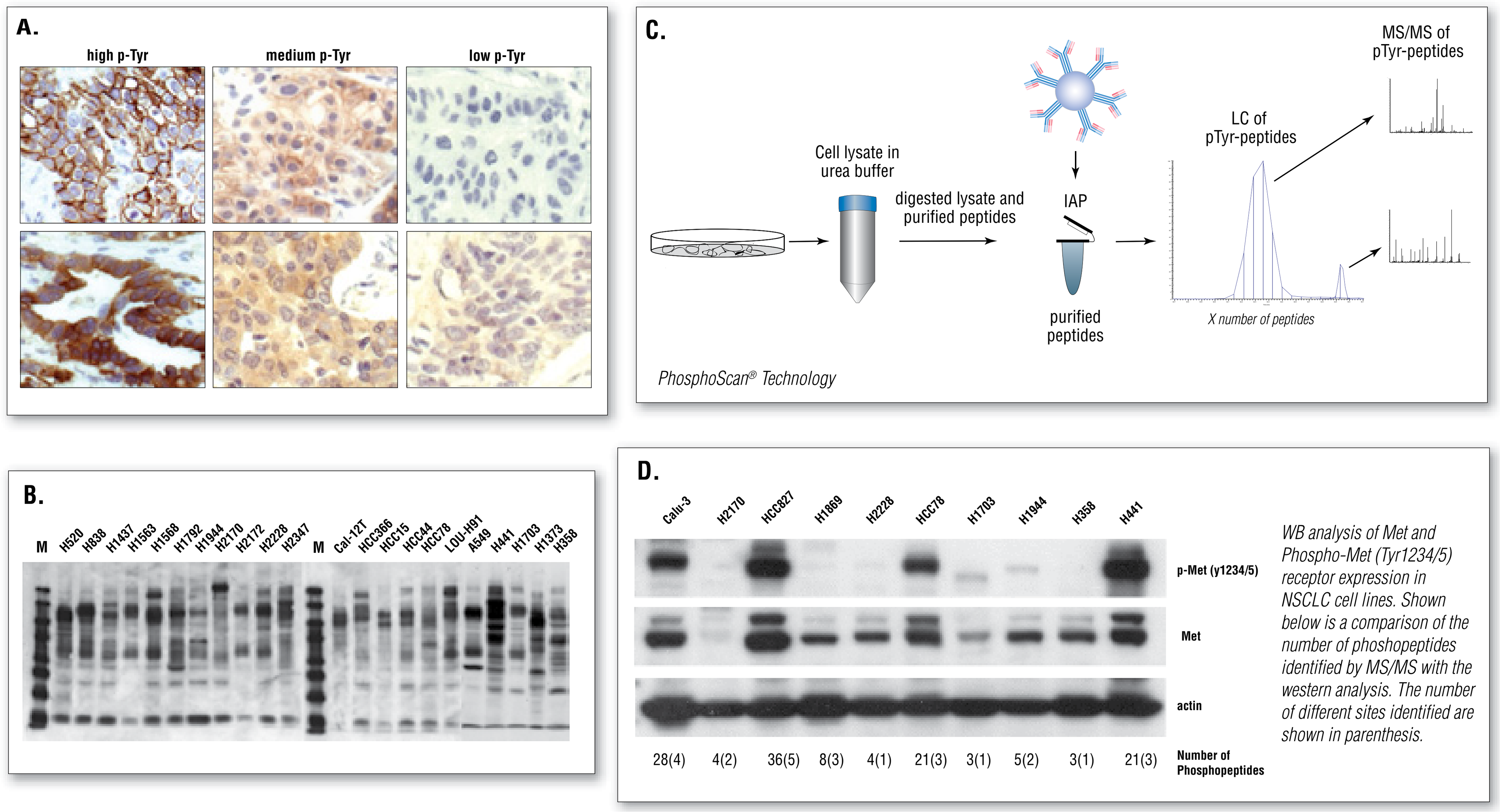


Figure 2. Classification of tyrosine phosphorylated proteins by gene ontology (A) in cell lines and tumors. Classification of phosphorylated RTK's (B), and cytoplasm tyrosine kinases (C) from cell lines and primary patient samples.

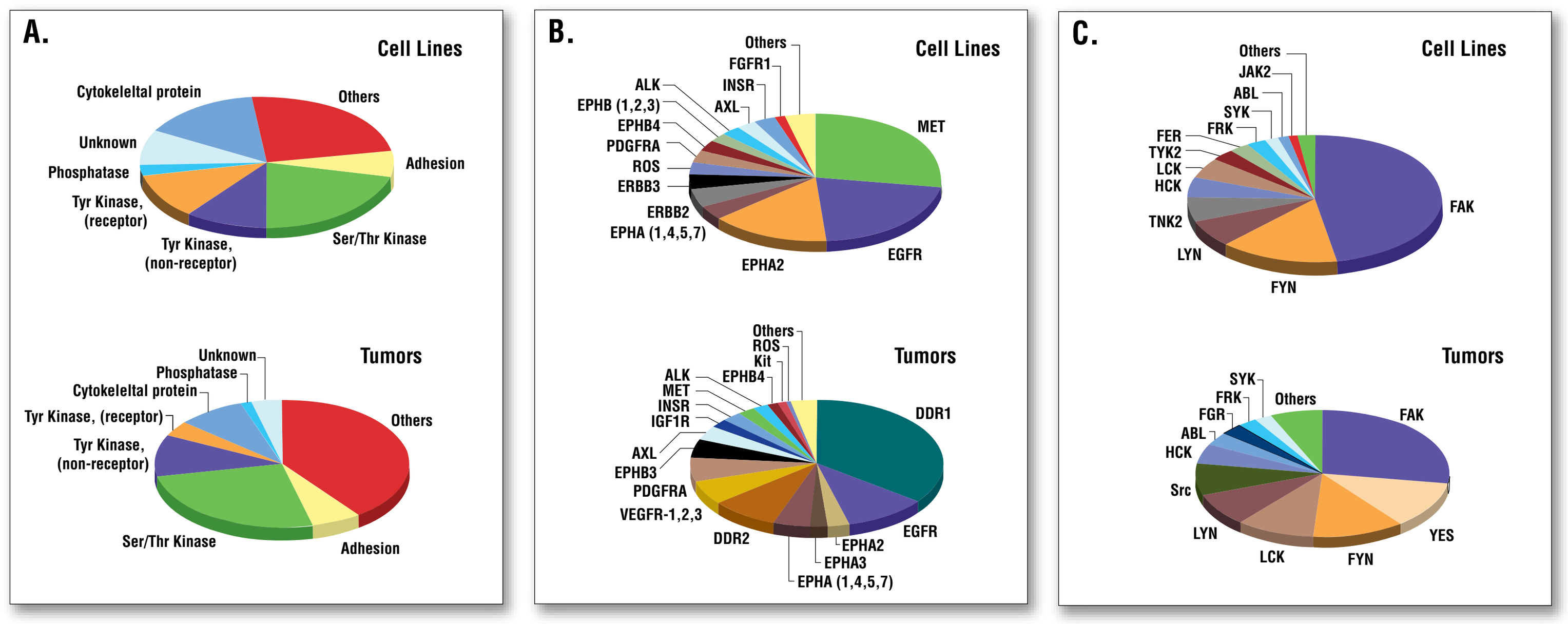


Figure 3. Results of unsupervised hierarchal clustering of 50 most informative proteins showing the highest SD across the 41 NSCLC cell lines before (left) and after subtracted background (right).

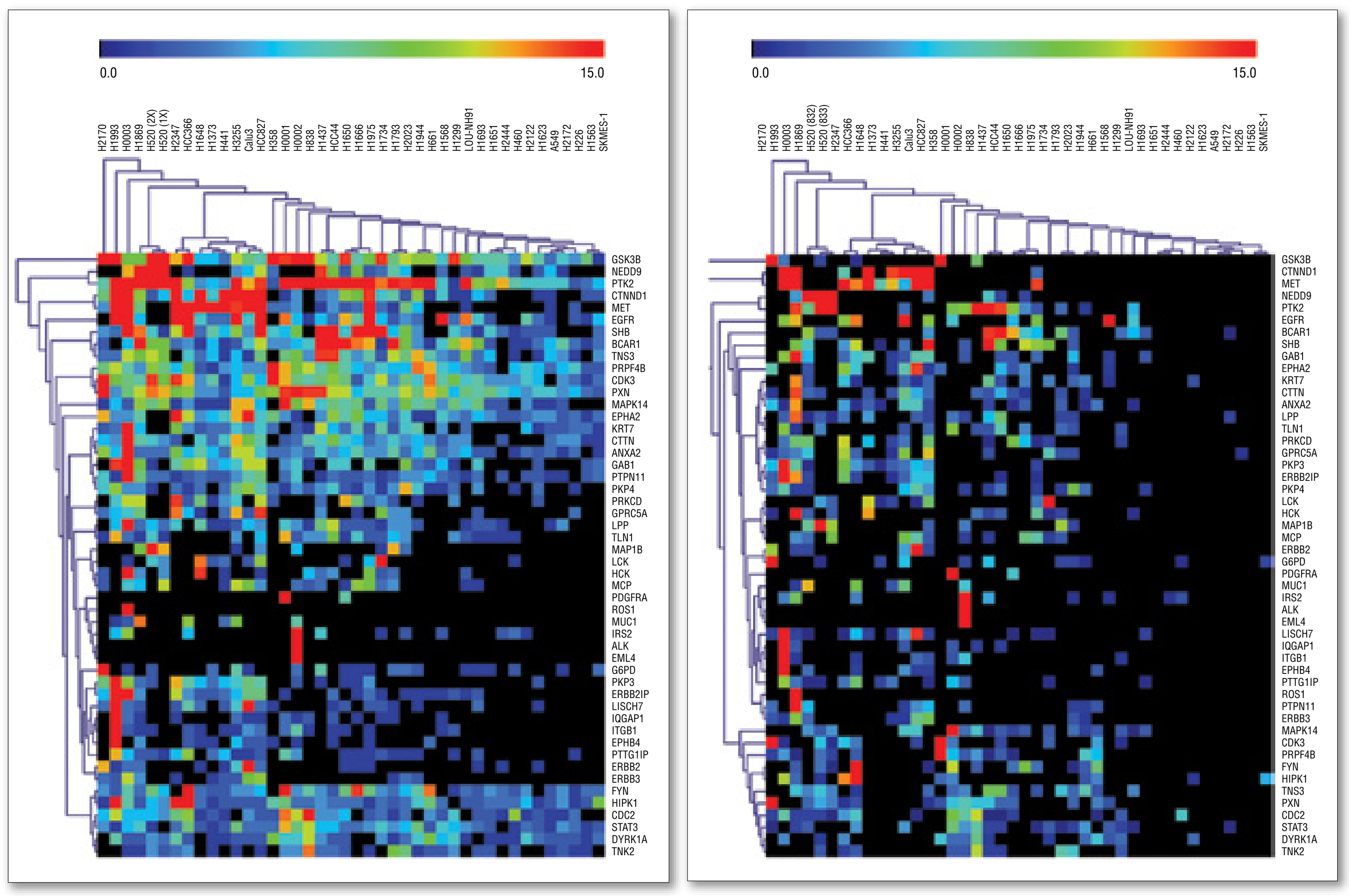


Figure 4A & 4B. Unsupervised hierarchal clustering of phosphorylated receptor tyrosine kinases in cell line (A) and primary tumors (B).

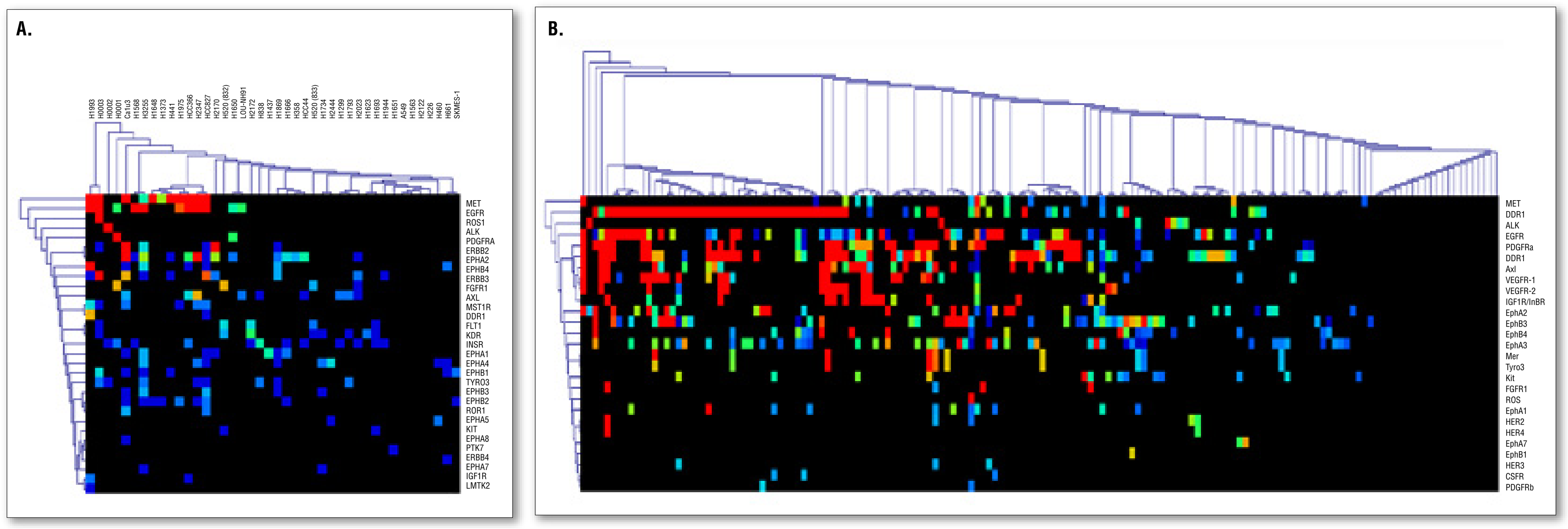


Figure 4C–G. Selected subgroups of most deregulated tyrosine kinases EGFR, Met, PDGFRα, ALK, and ROS in NSCLC.

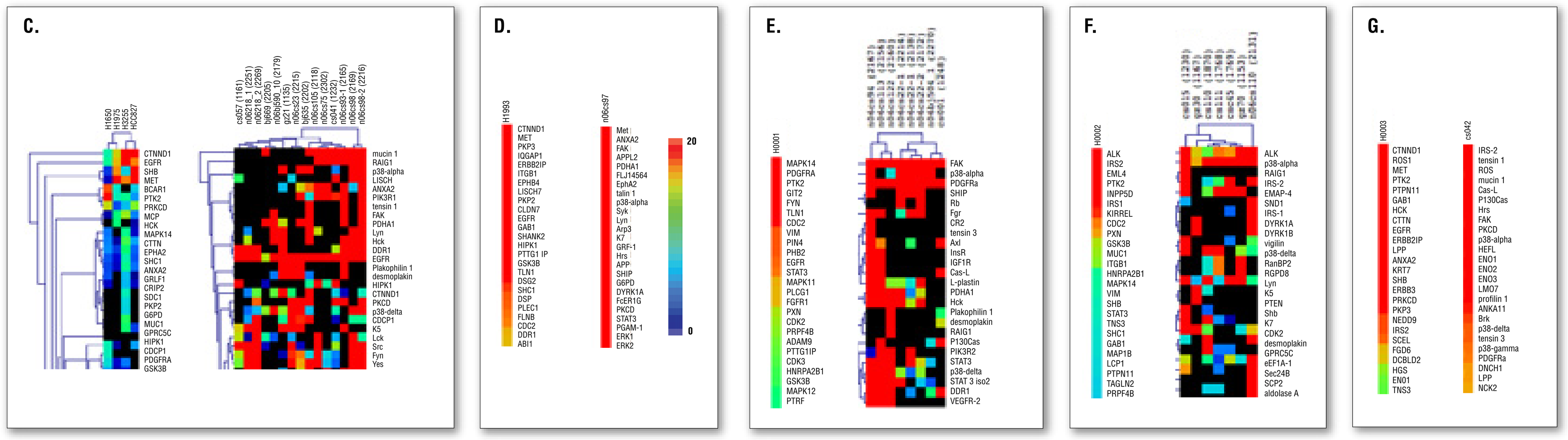
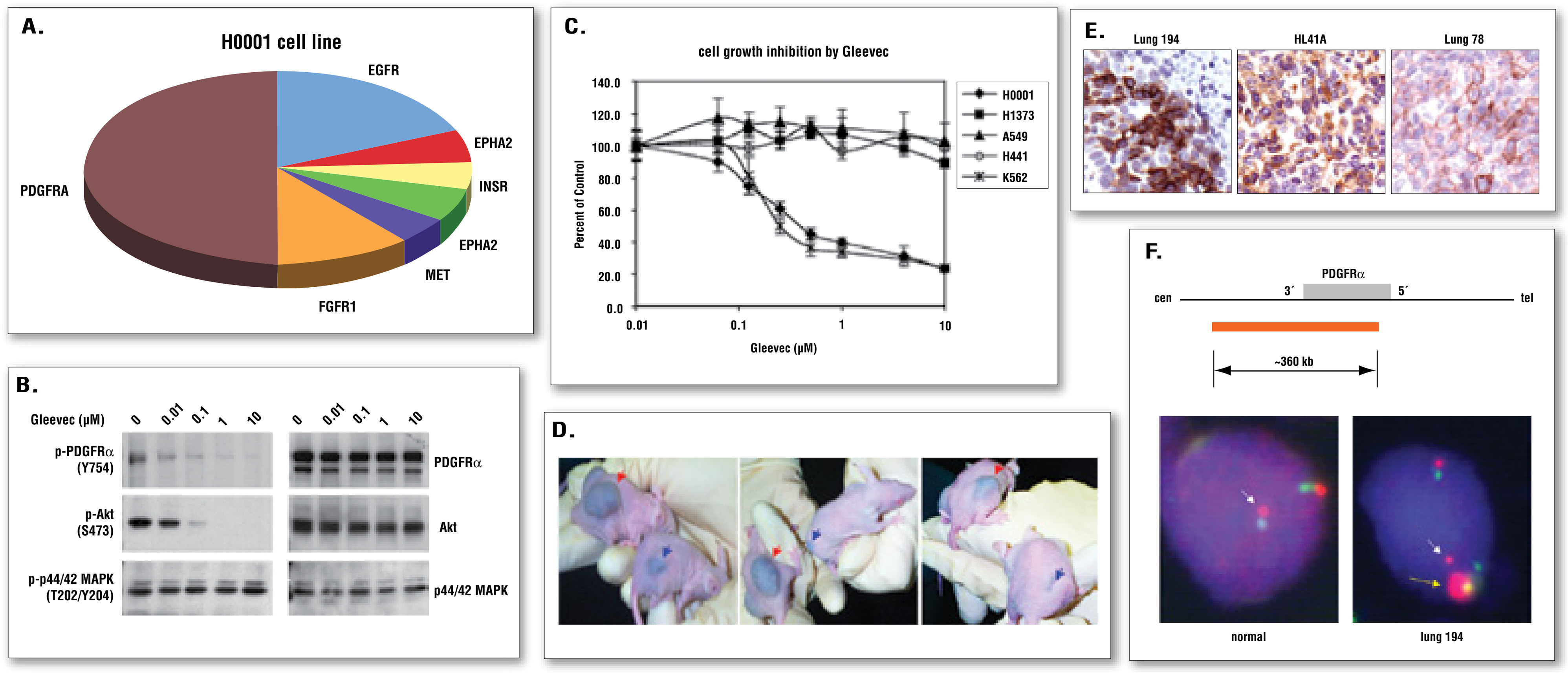


Figure 5. Cell line and xenographs express active PDGFRα and are sensitive to Imatinib mesylate (Gleevec). IHC staining of PDGFRα, and FISH results of PDGFRα in NSCLC patient samples.



Conclusions

- First large scale unbiased survey of tyrosine kinases activity in lung cancer.
- Known and novel PDGFRα, Ros and Alk deregulated tyrosine kinases activity is identified.
- Similar signatures of deregulated tyrosine kinase activity are observed between cell lines and tumor samples–suggesting cell lines are good models.
- We identified a group of aberrantly active receptor and non-receptor tyrosine kinase among the 154 tumor samples as candidate disease "drivers".

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