

MUTATIONAL SCREENING IN TYROSINE-KINASE DOMAIN OF *erbB1* AND *erbB2* GENES

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3.1 Introduction

The protein-kinase family is the most frequently mutated gene family found in human cancer. The Epidermal Growth Factor Receptor (EGFR or *erbB1*) and the Receptor Protein-tyrosine Kinase *erbB2* (HER2, Neu, *erbB2*) are transmembrane glycoproteins with intrinsic tyrosine kinase (TK) activity. Upon ligand binding, those TK-receptors are autophosphorylated, serving as a high affinity-binding site for cytosolic substrates, thus transducing downstream signals required for cellular responses. In this way, the TK-protein family has been suggested to have a role in cancer development and progression. Thus, aberrant expression and activation of EGFR/*erbB2* tyrosine kinase domains has been implicated in several key aspects of human neoplasia such as increased proliferation, survival, and invasiveness of cancer cells (Nicholson et al.2001). For example, abundant expression of EGFR has been detected in Non Small Cell Lung Cancer (NSCLC) human tumor specimens (Nguyen et al.2004) and abundant expression of *erbB2* has been detected in Breast Cancer (BC).

Based on these multiple impacts on cancer cell physiology, the EGFR and *erbB2* tyrosine kinases domains have been recognized as an attractive molecular target for selective treatment of solid tumors with increased TK activity levels. Thus, oral small molecule inhibitors of EGFR-TK domain, gefitinib and erlotinib, were approved for the treatment of patients with NSCLC (Lynch et al.2004) (Tracy et al.2004). Those works have indicated that the presence of somatic mutations in the tyrosine kinase domain of the EGFR is a major determinant of the response to the tyrosine kinase inhibitors gefitinib and erlotinib. Lynch et al. proposed that a subgroup of patients with NSCLC have specific mutations in the EGFR gene, which correlate with clinical responsiveness to the

TK-inhibitor gefitinib; these mutations lead to increased growth factor signaling and confer susceptibility to the inhibitor.

However this is still unclear and further work is needed to identify the factors underlying the response in those who do not carry mutations (Speake et al.2005).

3.2 Materials and Methods

In my stage at the Human Cancer Genetics Program, Comprehensive Cancer Center (Ohio State University), I participated in the EGFR and *erbB2* tyrosine kinase mutational analysis study whose aim was to determine the biological basis for differential responses to tyrosine kinase inhibitors (Weber et al. 2005).

The TK domain of EGFR, encoded by exons 18-21, and the TK domain of *erbB2*, encoded by exons 19-24, were directly sequenced in NSCLC tumor specimens. Genomic DNA was extracted by proteinase K digestion method.

PCR consisted of 40 cycles using an annealing temperature of 55°C in a 15 µl reaction mixture containing 7.5 µl HotStar MasterMix, 1.5 µl 5xQ-solution (Invitrogen, Carlsbad, CA, USA) and 0.25 µl of each primer (listed below). Primers were designed by Primer3 software (frodo.wi.mit.edu/cgi-bin/primer3). PCR products were then sequenced using Big Dye v3.1 terminator technology and the ABI 3730 analyzer (Applied Biosystems, Perkin-Elmer Corp., Norwalk, CT, USA) according to the manufacturer's recommendation for mutation analysis.

Primers for mutation analysis in EGFR gene: exon 18 sense GCTGAGGTGACCCTTGCTC; exon 18 antisense ACAGCTTGCAAGGACTC TGG; exon 19 sense CATGTGGCACCATCTACA; exon 19 antisense CAGCTGCCAGACATGAGAAA; exon 20 sense CACACTGACGTGGCCTC TCC; exon 20 antisense TATCTCCCCTCCCCTATCT; exon 21 sense CCTCACAGCAGGGTCTTCTC and exon 21 antisense CCTGGTGCAGGA AAATGCT.

Primers for mutation analysis in *erbB2* gene: exon 19 sense GATCTCCTGGAAGGCAGGTA; exon 19 antisense AGGTCTGGGCTCTGGTC TCT; exon 20 sense CTCTCAGCGTACCCTTGTC; exon 20 antisense CCTAGCCCCTTGTCACATA; exon 21 sense CAGAAGGTCTACATGGGT GCT; exon 21 antisense TTCTCCCATGGGTAGACAC; exon 22 sense CTCCCCACAACACAGTTG; exon 22 antisense AGCTCTATCCTCCCTC CAG; exon 23 sense

CTCCCACTCCTGACCCTGT; exon 23 antisense
AGGACCTCCCACCCTCCT; exon 24 sense
AGAGGCAGCAAGCACACAG and exon 24 antisense
GAGGGTGCTCTTAGCCACAG.

3.3 Results

In EGFR gene, somatic in-frame mutations were detected 3.3% of the NSCLC samples. The mutations consisted of 2 in-frame deletions who were detected in exon 19, around the ATP-binding pocket of the tyrosine-kinase domain. Although Lynch et al. proposed the screening for mutations in EGFR-TK domains to identify lung cancer patients who will have response to gefitinib, current data suggest that mutational spectra may be only one criteria for prediction of response to EGFR-TK inhibitors, among other criterias that may be considered (Weber et al.2005).

References

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