DETECTION OF EGFR GENE MUTATION AND GENE AMPLIFICATION IN COLORECTAL CANCER

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BACKGROUND: EGFR mutations in exons 19 ~ 21 have been correlated with clinical response to tyrosine kinase inhibitor (TKI) therapy in lung cancer. The mutations of exon 19 (deletion of 15 nuclotides; del 746-750) and exon 21 (CTG→CGG; L858R) are associated with sensitivity to TKI therapy, whereas the mutation of exon 20 (ACG →ATG; T790M) is related to resistance to TKI treatment. The presence of EGFR tyrosine kinase mutations in metastatic colorectal cancer (mCRC) has been reported with a few sequencing results and concluded as insignificant in monoclonal anti-EGFR therapy. However, DNA sequencing using PCR methods is not an optimal clinical assay for gene mutations. We develop a screening method of the real-time PCR melting temperature (Tm) analysis using LightCycler 2.0 for the mutations in EGFR exons 19-21 and the fluorescence in situ hybridization (FISH) for the EGFR gene amplification. Seventeen archived CRC samples with no treatment are investigated. MATERIALS AND METHOD: The genomic DNA was extracted from paraffin sections using the DNeasy Blood and Tissue Kit (QIAGEN Inc, Valencia, CA 91355). The presence of EGFR exons 19-21 mutations was analyzed by real-time PCR with the mutation-specific hybridization probes manufactured by TIB Molbiol (Berlin, Germany). The EGFR FISH assay was carried out in sections cut at 4μm from paraffin-embedded tissue blocks, using the LSI EGFR/CEP 7 DNA Probe set (Abbott Molecular Inc, Des Plaines, IL 60018). RESULUS: In the detection of exon 19 mutations, a few most common deletions (-9, -15, -16, -19nt etc.) were tested in the pilot study but only del 746-750 had the consistent results. Against the del 746-750 specific probe, no mutations was found in 17 CRC patients. In EGFR exon 20, the T790M mutation was not found, but its upstream common polymorphism-Q787Q (no change of amino acid) was sequenced in 12 of 17 CRC patients. In EGFR exon 21, the L858R mutation was not yet detected. All results were confirmed by sequencing. Results of FISH demonstrated no amplification of the EGFR gene (the ratio of EGFR to Cep7 >2.9), but eight cases of polysomy (>3 copies of Cep7) in 17 CRC patients. CONCLUSIONS: The real-time PCR Tm analysis using LightCycler is a fast and sensitive qualitative assay for the detection of EGFR mutations. Somatic mutations in the kinase domain of EGFR (exons19-21) are absent or very rare if present in colorectal cancer, despite the well-known abundance of EGFR protein expression. Increase in EGFR gene copy number although has been indicated as cetuximab responder, is a rare event in mCRC. In contrary, polysomy predominant in mCRC would be a better predictive element.