P3.03

AMPLIFICATION OF SINGLE NUCLEOTIDE POLYMORPHISMS OF THE EGFR GENE PROMOTER SEQUENCE IN NSCLC PATIENTS

Vladimir Jurisic, Jasmina Obradovic

University of Kragujevac, Kragujevac, Serbia

Epidermal growth factor receptor (EGFR) is an important regulator of tumor growth and metastasis to be overexpressed in many tumours including NSCLC also. Based on variation in EGFR gene, not all NSCLC patients respond equally to this therapy. Therefore, personalisation of drug therapy in NSCLC patients requires genotyping of EGFR gene. The aim of this study was to test the effects several additives in various concentrations for amplification single nucleotide polymorphisms in promoter sequence of the EGFR since the promoter sequence containing the multiple GC regions which are difficult for the amplification. PureLink™ Genomic DNA Kits (Invitrogen/ Life Technologies, Carlsbad, CA) were used for extracion of DNA from formalin-fixed paraffin-embedded lung cancer tissue. EGFR polymorphisms -216G>T/ and -191C>A were genotyped using the PCR-RFLP method. Sequencing was conducted using ABI PRISM® BigDyeTM Terminator v 3.1 Cycle Sequencing Kit in both forward and reverse direction. Resultes showed that between several tested additives including; glycerol, DMSO, formamide, Tween 20, Triton X-100, PEG and BSA, only a two have effectiveness including glycerol and DMSO, and with best results at concentrations of 15% and 5% respectively. Comparison of the obtained sequence with the reference sequence of EGFR promoter region (http://www.ncbi.nlm.nih.gov; GenBank reference: M11234.1) revealed that the PCR amplification was highly specific. We have shown that using appropriate co-solvent it is possible amplify promoter region of EGFR for single nucleotide polymorphisms -216G>T or -191C>A, that contained multiple GC regions.