Abstract No.234

Mutation Analysis of Tumor Suppressor Gene PTEN in a Random Population of Iranian Patients with Gastric Cancer

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PTEN (phosphatase and tensin homolog deleted on chromosome 10) is a tumor suppressor gene that encodes a dual-specificity protein phosphatase which contributes to regulation and propagation of signal transduction through the PI3K/AKT signaling pathway.The defects in this gene, for example mutations and deletions, are responsible for the development of some advanced cancers including endometrial cancers, ovarian cancers, and glioblastomas.The aim of this study is to investigate the significance of PTEN gene mutations on occurrence and development of gastric cancer.Exon 5 and 7 of PTEN gene were screened for mutations by " PCR-SSCP-DNA sequencing" followed by silver staining in 94 and 50 patients respectively with pathologically proven gastric carcinoma.No mutations were found in the regions mentioned. Our initial results suggest that there is no significant correlation between mutation in exon 5 and 7 of PTEN gene and occurrence and development of gastric cancer in Iranian patients. But, the exact results need further analysis.

Keywords: PTEN, Tumor Suppressor Gene, Gene Mutation, Gastric Cancer.

Abstract No.235

Studying of ATBF1 Mutations in a Random Population of Iranian Gastric Cancer Patients

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AT motif-binding factor 1 (ATBF1) is a transcription factor on 16q22 region with tumor suppressor activity that contains 4 homeodomains and 23 zinc-finger motifs . ATBF1 was identified as a transcription factor that binds to an AT-rich region, known as the AT motif in the α-fetoprotein gene promoter region.This transcription factor, down regulates AFP gene expression through binding to the AT motif competitively with hepatocyte nuclear factor 1(HNF1) .ATBF1 protein is known to interact with various proteins including c-Myb and PIAS3. ATBF1 represses c-Myb transcription activity through protein–protein interactions, which may in turn result in the suppression of cell growth and differentiation. Moreover, ATBF1 cooperates with p53 to activate the p21Waf1/Cip1 promoter and trigger cell cycle arrest. α-Fetoprotein (AFP) producing gastric cancers are aggressive tumors with venous and lymphatic invasion and hepatic metastasis. The goal of the present study is to investigate whether somatic changes in the AT motif binding factor-1(ATBF1) gene in exon 9 and 10 and LOH analysis with two microsatellite markers D16S3066 and D16S3139 in the development or progression of gastric cancer. Until now we have searched for allelic loss (LOH) with the microsatellite marker D16S3066 at the ATBF1locus by single-strand conformational polymorphism (SSCP) and sequencing methods in tumor and blood samples of 14 different patients with gastric cancer. We haven't found any LOH in tumor samples yet. However, we need to study more samples to investigate the correlation between progression of gastric cancer and allelic loss in this region in Iranian patients.Methods: DNA extraction from blood and tumor tissues, PCR of DNA samples, SSCP of PCR products and sequencing of DNA samples.

Keywords: AT motif-binding factor 1(ATBF1), D16S3066, D16S3139, Gastric Cancer.

Abstract No.236

Erlotinib-Protein Binding: HPLC Method Development and Validation

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Lung cancer is the leading cause of cancer-related mortality worldwide, for both men and women. Erlotinib a reversible tyrosine kinase inhibitor, which acts on the epidermal growth factor receptor (EGFR) is almost a new drug used for the treatment of non-small cell lung cancer after the failure of more than one or two courses of previous chemotherapy. The binding of drug to plasma proteins can influence its action and pharmacologic response. Therefore protein binding studies would be of great importance from the clinical point of view. In the present study a simple and rapid reversed-phase high performance

liquid chromatographic (HPLC) method with UV detection at 330 nm was developed for detection of dialyzed Erlotinib in protein-binding studies in the presence of albumin. Equilibrium dialysis method with fast spin dialyzer and 25 KD Nitrocellulose filters were used. A reversed-phase Symmetry C18 column (250 mm x 4.6 mm, 5 µm) was used at room temperature. The mobile phase was a mixture of methanol, acetonitril and potassium dihydrogen. Analysis was run at a flow rate of 1.3 ml/min. The run time for Erlotinib was approximately 7 minutes. The method was validated for its specificity, linearity, accuracy and precision.Therefore a simple, accurate and precise reversed-phase isocratic HPLC method with UV detection has been optimized and validated for the determination of erlotinib in human plasma.

Keywords: Erlotinib, Protein-binding, HPLC, Plasma.

Abstract No.237

Interaction of Erlotinib Hydrochloride with Human Serum Albumin

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Erlotinib hydrochloride is a drug used to treat non-small cell lung cancer (NSCLC), pancreatic cancer and several other types of cancer. Erlotinib specifically targets the epidermal growth factor receptor (EGFR) tyrosine kinase, which is highly expressed and occasionally mutated in various forms of cancer. The human serum albumin (HSA) is a major plasma protein. It is named a multifunctional plasma carrier protein because of its ability to bind to an unusually broad spectrum of ligands. HSA binds to the number of drugs altering their pharmacokinetics. Thus, the interaction between erlotinib hydrochloride as a drug with human serum albumin is significant. In this study, experimental investigation on the interaction of erlotinib hydrochloride with human serum albumin was carried out.Stern–Volmer dynamic quenching constant, binding constant and the number of binding sites for interaction of erlotinib hydrochloride with HSA were measured using analyzing of the fluorescence spectroscopic data. The intrinsic fluorescence spectra indicated that the intensity of fluorescence emission decreases as a function of erotinib concentration indicating the partial opening of the protein structure upon interaction with the drug. Fluorescence measurements on HSA–ANS complex was carried out to give information on the variations of HAS accessible hydrophobic areas and compactness of the protein. The increase in ANS emission indicated that the drug affects on the hydrophobic accessible surface area of HAS and leads to exposing of its hydrophobic groups.

Keywords: Erlotinib, HSA, Interaction, Spectroscopy.

Abstract No.238

Rolling Circle Amplification Technique in Medical Diagnosis

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Due to its robustness and simplicity, the Rolling-Circle Amplification (RCA) of circular DNA probes holds a distinct position in DNA based diagnostics among other isothermal detection methods. RCA reactions exhibit an excellent sequence specificity that is favorable for genotyping or mutation detection, antigen detection, DNA based biosensors and allows to unambiguously identification of DNA markers on the excessive unrelated background. We use circular DNA as a template for RCA reaction. When primers anneal to the template in presence of nucleotides, reaction buffer and phi29 DNA polymerase, RCA proceeds and make long strand DNA. In the presence of two primers, a complex pattern of DNA strand displacement ensues that generates 109 or more copies of each circle in 90 minutes. Using a single primer, RCA generates hundreds of tandem linked copies of a covalently closed circle in a few minutes. An important factor for the success of this method is the unique nature of phi29 DNA polymerase which has excellent strand displacement activity. The use of primers with 3´ thiophosphate-protected ends is also important, allowing circular DNA molecules to be amplified at least 10,000-fold by protecting the primers from the 3´_exonuclease activity of phi29 DNA polymerase. To achieve amplification, phi29 DNA polymerase appears to initiate multiple replication forks on each circle and to perform exponentially cascading strand displacement amplification. These results indicate that circular DNA probes can be amplified to the high levels required for solution based DNA diagnostics.

Keywords: Rolling Circle Amplification, Phi29 DNA polymerase, Displacement Activity, Isothermal Amplification.
