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Multiple molecular markers nested PCR assay for sensitive and specific detection of circulating hepatoma cells: Enhanced detection of HCC

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Background: HCC is a multifactorial, multistep and complex process. Its prognosis is poor and early diagnosis and monitoring of metastasis of HCC is of the utmost importance. Circulating Alpha-Feto Protien (AFP) mRNA has been proposed as a marker of HCC cells disseminated into the circulation but the specificity of this molecular marker and its correlation with the main HCC clinic-pathological parameters remain controversial. In recent years, several different multi-markers assays have been developed for the detection of hepatoma cells in the peripheral blood of patients with HCC. In this study; we examined the expression of a combined multi-markers assay of cancer specific markers Melanoma Antigen Gene MAGE 1 and MAGE 3 mRNAs and liver specific marker AFP mRNA in blood specimens obtained from patients with primary HCC and also from non HCC patients and control group by nested reverse transcriptase polymerase chain reaction (RT- PCR) to offer a simple method with high sensitivity and specificity for detection of the circulating hepatoma cells.

Subjects & Methods: In this study 58 patients and 15 matched control subjects; the patients divided into four groups; group A; patients with primary HCC diagnosed by histology, imaging, serum AFP (n=32), group B; patients with cirrhosis with no evidence of HCC (n=12), group C; patients with metastatic cancer in liver (n=14) and group D; 15 healthy volunteer age and sex matched proved clinically and by laboratory investigations to be free from diseases as a control. The staging of HCC was carried out according to the (Tumor/Node/Metastasis) TNM classification.

Methods: Peripheral blood samples were obtained from all subjects; MAGE-1 and MAGE-3 mRNAs and AFP mRNA were detected by nested RT-PCR.

Results: The positive rates of MAGE-1, MAGE-3 mRNAs and AFP mRNA were 18/32 (56.3%), 15/32 (46.9%) and 19/32 (59.4%) respectively in the primary HCC patients. In the cirrhotic group only 4/12 (33.3%) patients were positive for AFP mRNA, where in the metastatic group 5/14 (35.7%) and 4/14 (28.6%) were positive MAGE-1and MAGE-3 mRNAs respectively. When correlating the positive MAGE-1, MAGE-3 mRNAs and AFP mRNA to TNM clinical stages; tumor number and tumor size, only MAGE-1and MAGE-3 mRNAs were correlated ($P<0.05$). None of the clinical control samples show detectable levels of MAGE-1 and MAGE-3 mRNAs and AFP mRNA in their peripheral blood.

Conclusion: Our results indicate that a multi-markers nested RT-PCR assay with cancer-specific markers such as MAGE-1 and MAGE-3 in combination with a hepatocyte-specific AFP marker may be a promising diagnostic tool for monitoring hepatocellular carcinoma patients. Since nested PCR utilizes a couple of internal primers to reamplify the specific PCR product, it exhibits higher sensitivity, stronger specificity and lower false- positive occurrence as compared to single RT.

Biography

Salwa Hassan Teama is currently working as an Associate Professor of Clinical Pathology at Faculty of Medicine, Ain Shams University, Egypt.

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