THE USE OF REAL-TIME QUANTITATIVE PCR TO DETECT PROSTATE SPECIFIC MEMBRANE ANTIGEN mRNA IN PATIENTS WITH PROSTATE CARCINOMA  D. Chu, Y. Liou, C. Chuang, R. Tzou, H. Lee, C. Sun. Chang Gung Memorial Hospital, Tao-Yuan, Taiwan.

Purpose: prostate specific antigen (PSA) has long been criticized for its lack of specificity in screening for the occurrence of prostate cancer. In this study, we tried to measure another biomarker level, namely the prostate specific membrane antigen (PSM) levels in the peripheral circulation from subjects suffering from either prostate cancer or benign prostate hyperplasia (BPH). Methods: Total RNA was extracted from 70 blood samples of prostate cancer patients and 19 of BPH cases. Reverse transcription was performed to convert mRNA to cDNA. The cDNA was analyzed with a novel real-time quantitative PCR protocol to measure PSM mRNA levels in the circulation. Melting curve analysis was adapted to assure the correct amplification data were obtained. Results: 41 of 70 prostate cancer patients showed positive results, while 9 of 19 BPH cases showed negative. Therefore, the sensitivity and specificity were determined to be 58.6 % and 47.4 %, respectively. For comparison, traditional nested PCR were performed to investigate whether the new method was superior. The sensitivity and specificity of nested PCR were determined to be 27.1 % and 57.9 %, respectively. The detection limits of these two methods were 0.0005 ng (for real-time quantitative PCR method) and 0.5 ng of PSM-cDNA (for nested PCR method). Conclusions: We have successfully developed a novel, non-invasive real-time quantitative PCR method to detect the PSM mRNA levels in circulations of prostate cancer subjects. This method might provide references for urologists diagnosing prostate cancer or monitoring patients' condition after treatment.


The gold standard for the diagnosis of bladder cancer is cystoscopy and direct biopsy. Especially in low-grade tumors the sensitivity is not satisfactory and up to half of the results may be false positive. Here we investigate whether a quantitative RT-PCR-based mRNA detection in urine could be a valuable alternative. Ki-67 mRNA was chosen as a marker since Ki-67 protein indices were shown to correlate with tumor grade and stage.

Preliminary results showed significant amounts of Ki-67 mRNA in urine of bladder cancer patients (n= 13) while no Ki-67 mRNA was detectable in the urine of healthy donors (n=8). We are in the process of collecting more patient samples including control groups. Actual results will be discussed with respect to specificity and sensitivity.

MEASUREMENT OF THYROGLOBULIN MESSENGER RNA BY REAL TIME QUANTITATIVE PCR  D. Li, A. Butt, S. Clark, R. Swaminathan. Department of Chemical Pathology, St Thomas’ Hospital, London, UK.

Follow-up of recurrent differentiated thyroid carcinoma involves the measurement of serum thyroglobulin (Tg). However, Tg auto-antibodies are present in a high proportion of thyroid carcinoma patients (up to 25%) and these can interfere with the Tg immunoassays. To overcome this obstacle, investigators have used real time quantitative RT-PCR for the measurement of Tg mRNA in the blood of patients with differentiated thyroid cancer, with varying degrees of success. In the present study we demonstrate the first reported use of the PAXgene(TM)Blood RNA collection tube and extraction kit method for the preparation of RT-PCR quality RNA with subsequent deployment of the latter in the development of a specific, sensitive and reproducible TaqMan® assay for the detection and quantification of thyroglobulin mRNA. Beta-actin mRNA was also assayed and results expressed as a ratio of Tg mRNA to beta-actin mRNA.

The intra-assay CV for Tg and beta-actin mRNA assay was 27.7% and 25.4%, respectively. Inter-assay CV was 20.8% and 28.8% respectively, for the two assays. Tg mRNA was detected in all cancer (n=42) and healthy (n=20) subjects. Tg mRNA in cancer patients was significantly higher than the healthy subjects (0.00169 ± 0.00013 vs 0.00051 ± 0.00015; p<0.0001). Fourteen cancer patients had detectable levels of serum Tg, and in these, Tg mRNA levels tended to be higher than in cancer subjects with undetectable serum Tg (0.00188 ±
DETECTION OF TUMOR-SPECIFIC mRNA IN CELL-FREE BRONCHIAL LAVAGE SUPERNATANT IN PATIENTS WITH LUNG CANCER

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Background: Bronchial mRNAs are a standard procedure in the workup of patients with suspicious pulmonary lesions.

Unfortunately the examination of the cytological material is often inconclusive. We wondered whether it is possible to isolate malignancy-associated mRNA from cell-free lavage supernatant.

Methods: Extracellular mRNA from cell-free lavage supernatant and serum of 16 patients with lung cancer (12 NSCLC, 4 SCLC) was isolated using a hot phenol method, reverse transcribed, and amplified by RT-PCR. The quantity and quality of the isolated RNA was checked after cDNA synthesis by amplification with β-actin specific primers. Afterwards a panel of 4 genes known to be expressed in lung tumors (hnRNP, pericentrin, aurora, PGP 9.5) was used for the detection of tumor-associated mRNA expression in lavage supernatant and serum.

Results: mRNA coding for β-actin could be isolated from lavage supernatant of 15/16 patients. The detection of tumor-related mRNA was possible in lavage supernatants from 6 patients and in sera of 2 patients. Pericentrin-specific mRNA was found in lavage supernatant of 4 patients, hnRNP-B1 coding mRNA in 3 patients and PGP 9.5 coding mRNA in 2 samples. mRNA coding for the STK 15 gene (aurora) was detected in 2 serum samples. Altogether 8/16 (50%) lung cancer patients could be identified with this method.

Conclusions: Intact mRNA can be isolated from cell-free lavage supernatant and its quantity and quality is sufficient for the detection of tumor-associated gene expression alterations. This might open new possibilities for a diagnosis of lung cancer.

SIMPLE METHODS FOR ISOLATION AND QUANTIFICATION OF NUCLEIC ACIDS IN BIOLOGICAL FLUIDS

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Simple methods for DNA and RNA quantification in biological fluids have been developed.

The procedure for nucleic acids isolation based on a guanidine thiocyanate/activated glass milk protocol was elaborated (Russian Patent 2002126328, RP200221343412). The procedure provides at least 85% isolation efficacy of 100 mers and 90-95% efficacy for longer polynucleotides free from substances interfering with the fluorescence assay.

The method for quantification of DNA and RNA is based on the determination of total concentration of RNA and DNA using SYBR Green II dye, measurement of DNA concentration using the Hoechst 33258 dye and calculation of the RNA concentration from these data. The method is based on our finding, that independent staining of DNA and RNA by the SYBR Green II dye, measurement of DNA concentration using the Hoechst 33258 dye and calculation of the RNA concentration from these data. The procedure allows for accurate determination of DNA concentration in the range 10-1000 ng/ml in the presence of 200-fold excess of RNA and determination of RNA concentrations in the range 10-1000 ng/ml in the presence of large excess of DNA (Morozkin E., Anal.Biochem. 2003, in press).

Time-resolved immunofluorimetric assay for DNA was developed using affinity purified Fab fragments of rabbit anti-double stranded DNA antibodies. Sensitivity of the assay is 8 pg/ml and the range 10-100,000 pg/ml DNA.

DNA in plasma of healthy donors and cancer patients is considerably underestimated by the immunofluorimetric assay due to masking DNA bound proteins. Dissociation of these complexes in guanidine thiocyanate solution allows for accurate estimation of RNA and DNA concentrations by the fluorescence-based method.

THE LEVEL OF Ki-67 SPECIFIC RNA IN URINE OF BLADDER CARCINOMA PATIENTS IS RELATED TO THE Ki-67 PROTEIN LABELLING INDEX OF TUMOR SAMPLES


The expression of the human Ki-67 protein is strictly associated with cell proliferation. Since the protein is only expressed in dividing cells the anti Ki-67 antibody MIB-1 has been widely used in histopathological studies to estimate the growth fraction of human neoplastic tissue samples in situ. For a variety of tumors including bladder carcinomas the fraction of Ki-67-positive tumor cells (the Ki-67 labeling index) has been proven to correlate with the clinical course of the disease. Using quantitative RT-PCR for Ki-67 RNA in urine extracts we show that the amount of Ki 67 RNA in urine is related to the Ki-67 labelling index in neoplastic tissue samples of patients with bladder carcinomas. Experiments have been performed to evaluate the potential of Ki-67 specific RT-PCR from urine samples for the detection of bladder carcinomas.

EXTRACTION AND AMPLIFICATION OF MRNA FROM THE CONDITIONED MEDIA OF CULTURED HUMAN CANCER CELLS


Background: A limited number of studies have indicated that it is possible to amplify extracellular mRNA from the serum of cancer patients. However, the number of serum specimens and the range of mRNAs analysed, to date, have been quite limited. It is important, therefore, to establish, in a well-controlled environment, if cancer cells routinely secrete gene transcripts detectable by RT-PCR.

Aim: The aim of this study was to (i) establish if it is possible to routinely extract and amplify extracellular mRNA from the conditioned media of cultured human cancer cells, (ii) compare methods of extracting the mRNA to find a reliable and reproducible method and (iii) investigate if any known tumour-related mRNAs are expressed and, if so, at what levels.

Methods: RPMI 2650 (human nasal carcinoma) paclitaxel (Tx) and melphalan (Ml) resistant cell lines were included in this study (Liang et al 2001). Flasks of these cells were set up in duplicate at a confluence of approximately 50-60%. Aliquots of the conditioned media were taken at intervals of 24 hours over a 96 hour period, passed through a 0.22µm filter, and were subsequently stored at -80°C. Total RNA was extracted from 100 µl of the conditioned media using either (i) The SV Total RNA Isolation system (Promega) or (ii) Tri Reagent (Sigma). RNA was also extracted from the corresponding cultured cells to be used as positive controls for all RT-PCR experiments.

Results: The message for beta-actin was amplified in all of the conditioned media samples, which confirmed the presence and integrity of the RNA samples. Gene transcripts for CK-19, HnRNP B1 and MDR1 were also detected. Extraction of extracellular RNA using Tri Reagent proved to be reliable, repeatable and easy to scale-up. Conclusion: The results from this study indicate that tumour cells secrete gene transcripts. This study also opens up scope for the use of in vitro systems to perfect techniques without the need to waste valuable clinical samples.

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