Identification of Tumour Markers from Publicly Available Gene Expression Data

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Published in:
Default journal

Publication date:
2003

Identification of Tumour Markers from Publicly Available Gene Expression Data

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Better tumour markers are required to assist prediction of the primary site in patients presenting with adenocarcinoma of unknown origin, to provide prognostic information and guide clinical management. Publicly available large-scale mRNA expression profiles from adenocarcinomas of breast, colon, lung, ovary, pancreas, prostate and stomach were downloaded from the website of the National Centre for Biotechnology Information (NCBI). Hierarchical clustering of this Serial Analysis of Gene Expression (SAGE) data showed that adenocarcinomas and their metastases cluster according to their site of origin and that these patterns are maintained during the metastatic process. A novel bioinformatic approach to identify the differences between primary sites was developed and used publicly available data generated from a range of technologies, including SAGE, digital differential display and microarrays. 61 candidates emerged, including the well-characterised tumour markers PSA and CEA, the identification of which supports the approach. Genes not previously regarded as tumour markers, including lipophilin B, were also highlighted. The expression patterns of these genes have been validated by RT-PCR in primary adenocarcinomas from the seven primary sites, and two-thirds of the candidates show tissue-restricted expression in concordance with bioinformatic predictions. Further testing is now underway in tissue microarrays of 350 adenocarcinomas. Investigation of public gene expression information is a powerful method for the identification of clinically relevant molecular markers.

Global cDNA Amplification of Formalin-Fixed, Paraffin-Embedded Tissues (FFPET)

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Genome wide gene expression profiling using microarrays is enabling novel methods of cancer classification and detection, with implications for understanding the pathobiology of cancer and improving treatment. Such methods rely on relatively large amounts of usually fresh, starting tissue, limiting their clinical application. We applied polyA RT-PCR to routinely processed human formalin-fixed paraffin embedded tissue (FFPET) in order to overcome these limitations.

Parallel fresh and formalin-fixed lymph node samples were obtained from Manchester Royal Infirmary, and total RNA extracted using Trizol reagent and an Ambion kit respectively. DNase was used to remove contaminating genomic DNA. Conventional reverse transcription was performed on the isolated RNA, and also polyA RT-PCR, using an oligo dT primer, 3' polyadenylation and multiple rounds of PCR with the same oligo dT primer. Both resultant cDNA samples were probed for GAPDH using specific 3' directed primers, verifying presence of amplified mRNA in both. Real-time PCR was used to compare the relative abundance of a range of genes in the frozen and fixed samples, and preliminary results suggest maintenance of the frozen sample gene expression profile in the fixed sample.

The results demonstrate the ability of polyA RT-PCR to amplify mRNA from FFPET. Since polyA PCR generates large amounts of cDNA, which is indefinitely renewable, this advance offers the potential of microarray gene expression profiling for routine clinical samples, and unlocks a vast archive for research.

The utilisation of a cDNA array scanner for quantitative fluorescent immunohistochemistry

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cDNA array scanners are highly sensitive, high resolution laser scanning instruments that enable the quantification of fluorescence from fluorescently labelled cDNAs hybridised to nucleic acid targets arrayed onto microscope slides. These scanners can also be used to investigate protein – antibody interactions using array formats. The instruments are able to scan and collect fluorescence data from microscope slides in six minutes at high resolution. We have investigated the potential use of this instrument for the quantification of fluorescent immunohistochemical reactions on whole paraffin sections and tissue microarrays.

To investigate the linear response of the scanner, a protein array was constructed by spotting known quantities of Cy3 and Cy5 labelled albumin and known quantities of a mixture of Cy3 and Cy5 labelled albumin onto Arrayit SuperEpoxy coated slides and Schleicher and Schuell 'Fast' nitrocellulose coated slides using a Microcaster manual arraying instrument. The slides were scanned at 5μm resolution in a dual laser Axon 4000B scanner and fluorescence was collected at 532nm and 635nm wavelengths. Data from the instrument showed a direct relationship between quantity of protein per spot and the fluorescence measured.

Sections of normal and tumour colorectal specimens were cut at 4μm and reacted with antibodies to p53, thymidylate synthase and SMAD4 using standard immunohistochemical procedures. Following reaction with the primary antibody the sections were incubated with a Cy5 labelled secondary antibody and counterstained with Sytox Green DNA stain. The slides were scanned and the fluorescence at 635nm was adjusted against the total DNA measured from the Sytox Green fluorescence collected at 532nm. A comparison between the adjusted 635 fluorescence in the normal and tumour showed either an increase or decrease in expression of the target protein. Parallel sections were also investigated by standard chromogenic immunohistochemistry to compare the quantification obtained between subjective scoring and the fluorescence result. The method was also applied to a tissue microarray constructed with cores from normal and tumour colorectal specimens. With a scan speed of less than six minutes, the method is ideally suited for the analysis of protein expression in tissue microarrays.

Cell Line Model of the Mitogen Activated Protein Kinase Signalling Pathway in Cutaneous Malignant Melanoma

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The importance of the mitogen activated protein kinase (MAPK) pathway in melanocytic naevi and melanoma has recently been revealed by identifying BRAF and RAS mutations in most tumours. However, it remains unknown whether MAPK degregulation remains essential in established tumours. This is important to know because inhibitors of this pathway are potential therapeutic agents. We assessed the effects of MAPK pathway suppression, via the inhibitor PD98059, on the behaviour of established melanoma cells using the melanoma cell line SK-MEL-28, which has a homozygous BRAF exon 15 activating mutation.

MAPK activity, cell proliferation and apoptosis were assessed on SK-MEL-28 cells treated with PD98059. Cell differentiation was observed after H&E staining; immunohistochemistry was used to measure MAPK activity, proliferation and apoptosis with antibodies against phosphorylated ERK1/2, Ki67, and active caspase-3 respectively. Invasion was assessed using an in vitro assay and RT-PCR for matrix-metalloproteinases.

The MAPK pathway was suppressed in SK-MEL-28 after treatment with PD98059, demonstrated by a 15 fold decrease in the level of activated ERK1/2 ($p < 0.025$). The proliferation index was reduced 10 fold ($p < 0.025$) and the cell morphology changed to a storiform appearance. Reductions were also seen in the invasion index and level of MMP-1 mRNA expression. No change in the apoptotic index was seen. In conclusion these findings show that MAPK activity plays an important role in malignant behaviour of the SK-MEL-28 cell line. A therapeutic strategy based on MAPK inhibition could be developed if other melanoma lines show similar responses. Further studies will be carried out on other melanoma cell lines with different BRAF or NRAS mutations for comparison.