TOWARDS DISCOVERY OF NOVEL TUMOUR MARKERS FOR HEAD AND NECK CANCERS

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Abstract

Recent advances in genomic technology, in particular gene expression profiling, may elucidate novel tumour markers or signatures that will predict how various tumours will behave and respond to various treatment modalities. In head and neck cancer, tumour markers may address current functional deficits in treating locally advanced disease. In the context of expression profiling, as achieved by microarray analysis of the relevant mRNA population, multiple studies have examined differences between normal epithelia and head and neck carcinoma. In this context, gene sets which might distinguish metastatic disease have been described. Gene profiling has also been correlated with clinical outcome and the work has been extended to characterise particular gene products as potential biomarkers. Such markers might be used to detect the presence of head and neck squamous cell carcinoma, metastasis of the cancer, or aid in determining the best treatment for the patient.

Head and neck squamous cell carcinoma (SCC) is among the top 10 most common cancers in Australia.[1] This group of malignancies and their treatment is often associated with marked morbidity and mortality, particularly in patients with locally advanced head and neck SCC.

A tumour marker can be described as any substance produced as a result of cancer growth. These tumour markers have established roles in other cancers for screening, diagnosis, prognosis, therapeutic monitoring and/or detecting recurrence. Well known examples include BRCA1/2, PSA and Her2/neu. These tumour markers can play a key role in tailoring treatment.[2-7] In head and neck cancer, such markers would be invaluable given the resulting functional deficits of treating locally advanced disease.[8-10]

With the completion of the sequencing of the human genome combined with advances in genomics and proteomics, there is a new potential to discover panels of novel tumour markers that may play an important role in the diagnosis/prognosis of head and neck cancers. Increasingly, research is examining patterns of gene expression or protein changes instead of elevated levels of specific tumour markers. These "molecular signatures" are established using genomic and proteomic techniques such as microarray analysis.

What is a microarray?

Microarrays, as the name suggests, are molecules or other small biological substances arrayed in a known, uniform order on a solid support. They can be broadly classified into three general groups: DNA, protein and tissue microarrays. DNA expression microarrays have been the most widely used to date. The development of expression microarray technology has allowed gene expression profiling at the RNA level to be conducted for tens of thousands of expressed genes simultaneously, by hybridising an array of known sequences with labelled cDNA reverse transcribed from the sample RNA. Expression profiling using DNA microarray analysis has been used for cancer classification[11-13] and prognosis-based treatment.[14] Other DNA microarrays designed to examine regions of chromosomal amplification or deletion, or chromosomal methylation are also widely used.

Protein microarrays are currently an emerging technology and are generally a piece of glass on which different molecules of protein have been affixed at separate locations in an ordered manner, forming a microscopic array.[15, 16] These may be used to identify protein-protein interactions, to identify the substrates of protein kinases, or to identify the targets of biologically active small molecules. The most common protein microarray is the antibody microarray, where antibodies are spotted on to the protein chip and are used as capture molecules to detect proteins from cell lysate solutions.

Tissue microarrays are paraffin blocks that contain tissues assembled in array to allow a large number of biopsies to be sectioned simultaneously for immunohistological analysis.[17] The "microblocks" are usually cored biopsies of tumour or clinical specimens of approximately 0.6mm in diameter. These tissue cores are inserted in another separate recipient paraffin block in a precisely spaced, array pattern. Numerous sections of many tissues can be taken for independent tests.[18] These are usually sectioned for immunohistochemistry or in situ hybridisation. Tissue microarrays are a rapid and convenient way to screen a number of tumour markers by antibody staining across a large number of patients.

Discovering novel markers in head and neck SCC

A large effort by many groups has been made to identify novel tumour markers in head and neck SCC over the

past few years. Many of the initial studies described global changes in gene transcription that distinguished normal head and neck squamous epithelia from carcinoma. Chin et al studied the common alterations in the transition from mucosa to primary tumour and regional nodes using matched autologous tissues respectively in over 13,000 genes.[19, 20] They found over 1200 gene products showing statistically significant differences in expression in the transition from normal oral mucosa to the primary tumour. Studies from other laboratories have also demonstrated grouping of transcriptional profiles that distinguished pre-neoplastic versus cancerous epithelium.[21] Patients with verrucous leukoplakia and erythroplakia, both premalignant conditions, were found to share a higher degree of relatedness to oral SCC samples than to normal controls. This phenomenon has also been observed by others and may suggest that changes in gene expression may occur before the development of malignancy, raising the hopes of developing tumour markers to detect very early-stage lesions.

More recent research has focused on the elucidation of gene expression profiles distinguishing metastatic disease from non-metastatic disease. Tumours of the oropharynx, hypopharynx and larynx have been found to group significantly according to metastatic cervical lymph node status.[22] A study evaluating the gene expression profiles of 34 hypopharyngeal tumour specimens identified a subset of 164 genes that were associated with metastatic potential, as indicated by patients with or without clinical evidence of metastasis three years after surgery.[23] Others have identified a 116 gene signature set that differentiated primary tumour specimens according to metastatic lymph node status, and showed that tumour specimens from lymph node metastases were similar to lymph node-positive primaries.[24] These authors went on to use the identified gene signature to "predict" the presence of lymph node metastases in a number of patients who were not included in the original data analysis.

A very recent series of studies by Roepman and colleagues has expanded on the metastasis predictor gene expression signature in head and neck SCC. These authors examined expression profiles from 82 head and neck SCC tumour specimens (45 metastatic and 37 non-metastatic) of the oral cavity and oropharynx and established a predictor set of 102 genes that was associated with metastasis. The performance of this predictor set was dependent on tumour tissue specimen storage times, exhibiting improving performance with shorter storage times. When the predictor set was assessed among expression profiles of 22 independent tumour samples, all stored for less than five years, lymph node status was correctly predicted in 86.4% of the tissue specimens.[25] Further analysis has shown that this initial gene set is part of a larger group of 825 genes,[26] with the suggestion that larger gene sets lead to more accurate predictions and are less prone to false negative calls. These findings taken together, suggest that there might be a metastatic gene expression signature present in some primary tumours that predisposes them to metastasise.

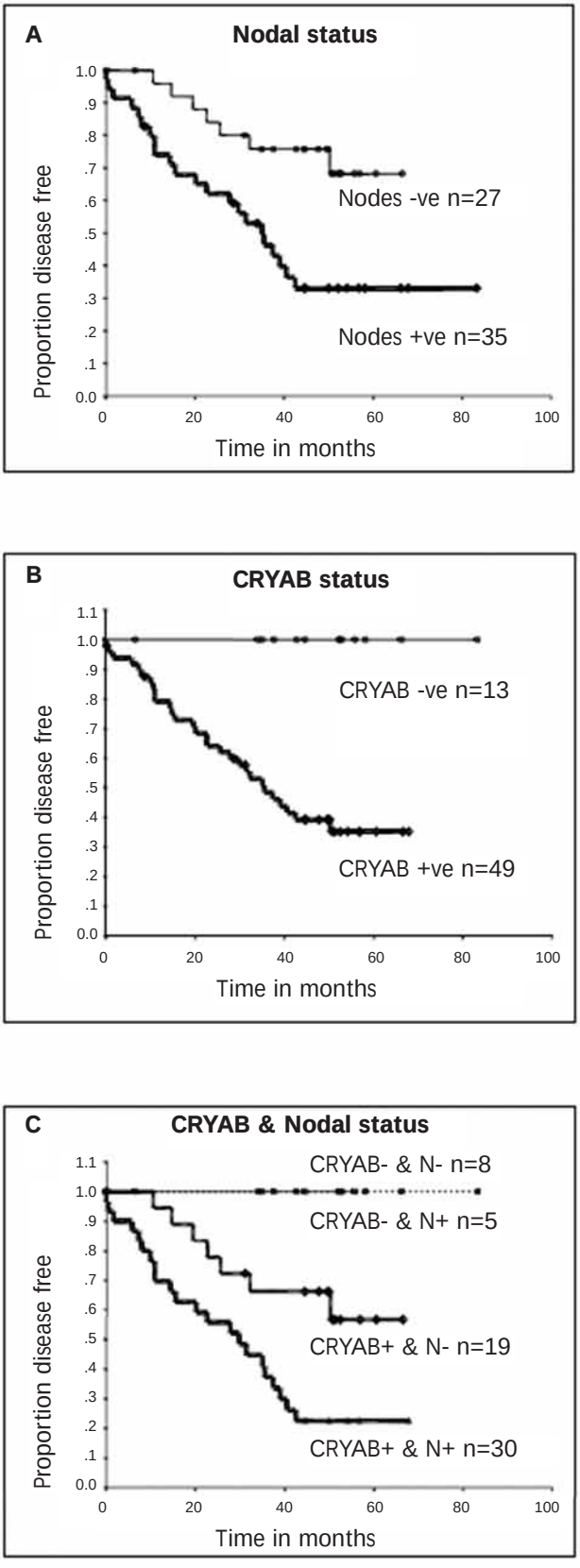

Figure 1

Kaplan-Meier survival curves. A. DFI with nodal status; Log rank P = 0.005. B. DFI with alpha-B crystallin (CRYAB) positivity; Log rank P < 0.001. C. DFI with alpha-B crystallin (CRYAB) and nodal status; Log rank P < 0.001. Taken from Chin et al.[28]

A great deal of research has also been conducted into attempting to correlate gene expression profiles from tumours with patient clinical outcomes. In an excellent study, Chung and co-authors identified gene signatures from tumours that clustered into four groups, which exhibited significantly different rates of disease recurrence-free survival.[22] Others have examined over 50 specimens from multiple sites and identified a set of genes with altered expression that grouped patients according to tumour recurrence, and therefore worse outcome.[27] A recent study from our laboratories has shown that elevated protein expression of one particular marker, osteonectin, was a powerful, independent predictor for short disease-free interval and poor overall survival in an independent group of 62 patients, following expression profiling of seven tumour specimens and autologous matched normal controls.[19]

These gene expression markers have the potential to become routinely used tumour markers. It may be possible to detect some or all of these changes by a simple biopsy or even a blood test. The pattern of alteration in these genes may be used as a diagnostic, prognostic and treatment modality indicator. However, many of the genes identified by the various studies are not well characterised and need to be studied functionally. There is also significant validation work required to correlate the changes in expression pattern with clinical outcome. In head and neck SCC, with most recurrence occurring within two years of treatment, it is possible to validate these gene expression changes in a retrospective study and correlating with clinical outcome.[19]

A simple test for a small number of changes however, would be technically easier and probably more widely used. Currently, our best marker alpha-B crystallin, the product of the CRYAB gene, is more sensitive than nodal status or tumour staging in determining disease free interval or overall survival (Figure 1). Tumours with no alpha-B crystallin present as judged by immunohistochemical staining do not develop recurrence regardless of nodal status.[28] This finding is currently being validated in a larger group of patients and to determine if head and neck SCC tumours negative for alpha-B crystallin staining are particularly sensitive to radiotherapy, as all of the nodal positive patients would have received this treatment.

Perspectives

One of the major criticisms of expression profiling studies to date, particularly those attempting to correlate or predict patient outcome, has been the lack of overlap of predicting genes between like studies. It is likely that the variation in tumour specimen characteristics could significantly impact this. With the development of more standardised techniques for sample preparation and data analysis, it is generally considered that these limitations will be overcome. Further, many have criticised the small patient numbers involved in these early studies. Clearly, larger studies of much larger sample sizes comprising tumour specimens of more uniform characteristics need to be undertaken. It is also crucial that any pattern or gene difference from expression profiling analysis be validated in an independent sample series to ensure the robust nature of the finding. Even with these current drawbacks, it remains possible to hope that some of the markers or patterns of markers identified in these studies could in the future be used to detect the presence of head and neck SCC, metastasis of the cancer, or aid in determining the best treatment for the patient. □

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