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Invited review DNA copy number changes as diagnostic tools for lung cancer

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ABSTRACT

Lung cancer usually presents as advanced stage disease and there is a need for early diagnosis so that appropriate treatments can be provided prior to tumour progression. Copy number variation is frequently detected in tumours and can contribute to tumour progression. This is because regions harbouring DNA imbalance can contain genes encoding critical proteins whose altered dosage contributes to the neoplastic process. Three copy number variations (CNVs) from chromosomes 3p26-p11.1 (loss), 3q26.2-29 (gain) and 6q25.3-24.3 (loss) have previously been described in individuals presenting with endobronchial squamous metaplasia. These CNVs were predictors of cancer diagnosed within 44 months with 97% accuracy. An evaluation of this CNV-based classifier with an independent set of 12 samples (10 men and 2 women), each with a carcinoma in situ or invasive carcinoma at the same site at follow-up demonstrated 92% prediction accuracy. The negative predictive value of this classifier was 89%. The gain at 3q26.2-q29 contributed the most to the classification, being present in virtually all lesions. This region harbours the PIK3CA gene and evaluation of the number of copies of this gene gave very similar results to those from array comparative genomic hybridisation. This type of test can be performed on sputum or bronchial brushings. Larger cohorts now need to be examined to confirm this finding and to possibly refine the regions of CNV. This type of approach paves the way for future molecular analyses to assist in selecting subjects with endobronchial squamous metaplastic or dysplastic lesions who might benefit from more aggressive therapeutic intervention or surveillance.

COPY NUMBER VARIATION CAN CONTRIBUTE TO TUMOURIGENESIS

The human genome is composed of approximately 3 billion bases and one-thirtieth of this encodes its ~23 000 genes. Changes in DNA content have occurred during evolution and include base pair alterations and gains and losses of segments of DNA (termed copy number variation (CNV)). Alterations in DNA copy number can also arise during the evolution of a tumour as a consequence of its inherent genomic instability. If these CNVs confer a selective advantage they can be maintained. It is likely that selection of a particular CNV is usually due to the presence of a single gene in the altered region. These genes include those critical in tumour initiation or progression, such as those affecting the vital processes of proliferation, resisting cell death, inducing angiogenesis, and activating invasion and metastasis. CNVs can also contribute to the tumour microenvironment.[1]

The size of CNVs in tumours varies from base pair changes to larger alterations comprising a gene fragment or one or several genes. It can also include gains or losses of whole chromosomes, chromosome arms or large genomic fragments. Losses usually involve tumour suppressor genes, whereas gains usually involve oncogenes. CNVs can predict tumour progression. For example, the HER2/*neu* and the epidermal growth factor receptor (EGFR) are significantly overexpressed in several cancer cells and account for the progression of many types of cancer: breast, ovarian, skin, pancreas and brain.[2] Gene amplifications usually lead to upregulation of the expressed gene, thereby upregulating the pathway they reside in.

The International Cancer Genome Consortium (ICGC) is a collaborative effort to characterise genomic abnormalities in 50 different cancer types.[3] Recent studies by large cancer consortia (eg, the Cancer Genome Atlas) have revealed large numbers of CNVs in a large number of tumour types. The ICGC also catalogue simple somatic mutations, structural rearrangements, gene expression changes, and alterations in microRNAs and DNA methylation. For example, a study of 178 lung squamous cell carcinomas recently revealed complex genomic alterations, and a mean of 360 mutations in exons, 165 genomic rearrangements, and 323 CNVs per tumour.[4]

The presence of a CNV can be used diagnostically, either to predict the presence of a cancerous cell or set of cells, or to predict tumour progression or response to treatment. It can also lead to the development of new tumour-fighting drugs. For example the anti-p185HER2/neu monoclonal antibody Herceptin (trastuzumab)[2] was developed in response to the observation that HER2/*neu* is amplified in some epithelial cancers. Metastases can also be accompanied by copy number alterations. For example, the development of colorectal cancer liver metastases is associated with amplification of chromosome 20q.[5]

METHODS FOR DETECTING COPY NUMBER CHANGES

The ability to detect a CNV depends on the size of the genomic alteration and the degree to which a DNA segment is altered with respect to copy number. Hybridisation of DNA from tumours and matched normal DNA to high-density arrays of DNA probes spanning the genome is one way of screening for CNVs. In the case of such arrays,

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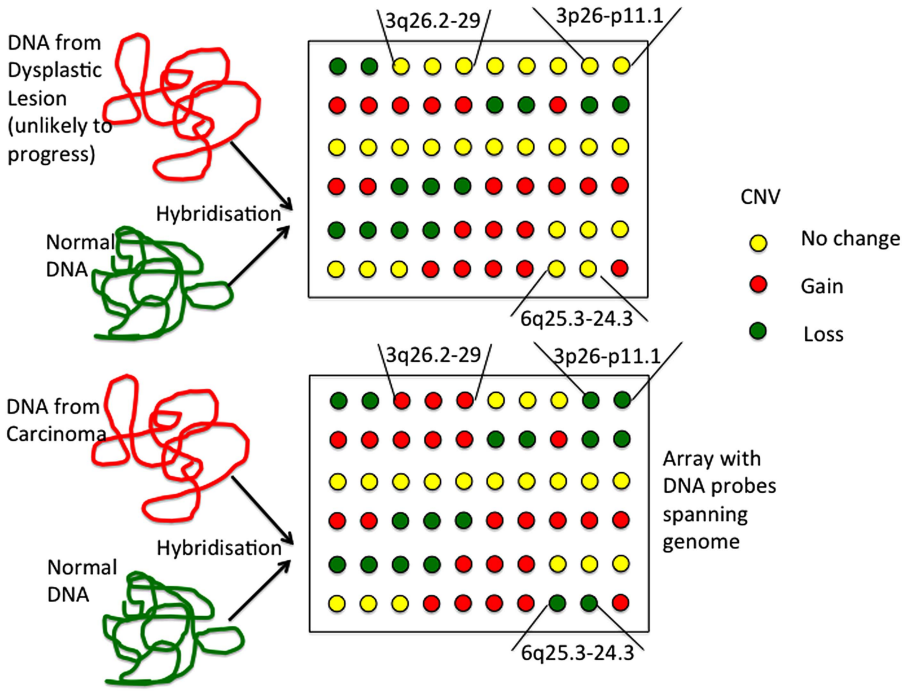

Figure 1 Diagram illustrating the results of array-based comparative genomic hybridisation for detection of copy number variations differentiating a dysplastic lesion unlikely to progress (upper) from that likely to lead to a carcinoma (below). In each instance DNA from the lesion is fluorescently labelled and then hybridised with matched normal DNA from the same patient (labelled with a different fluorescent dye). Copy number variation in the lesion likely to form a carcinoma at 3p26-p11.1 (loss), 3q26.2-29 (gain) and 6q25.3-24.3 (loss) is indicated as green, red and green overall fluorescence on the array (lower). Regions showing no difference between the lesion and normal DNA are indicated in yellow.

each probe corresponds to a single site in the genome and arrays can harbour millions of probes (figure 1). CNVs can then be detected by first labelling the tumour and the matched normal DNA with different fluorescent dye which can be represented by different colours (eg, green and red). The labelled DNAs from tumour and matched normal DNA are then hybridised together to the array. The amount of DNA from each sample that binds to each probe can then be evaluated on the basis of the overall fluorescence. Chromosomal gains in the tumour will be revealed on the basis of fluorescence that is primarily tumour specific (eg, red), whereas chromosomal losses will be revealed by fluorescence that is primarily specific to normal DNA (eg, green) (figure 1). No change in copy number can be revealed by intermediate fluorescence (eg, yellow). This technology is termed array comparative genomic hybridisation (aCGH) and has provided information on gains and losses of chromosomal segments for a large number of cancers.

CNV ANALYSIS FOR LUNG CANCER DETECTION

Lung cancer usually presents as advanced stage disease. Moreover, only few endobronchial squamous metaplastic and dysplastic lesions eventually progress to carcinoma (in situ). Hence, there is an urgent need for better diagnostic methods so that appropriate treatments can be provided. The accompanying paper by van Boerdonk and colleagues describes an attempt at early detection with molecular approaches. These authors had previously used aCGH to identify three CNVs from chromosomes 3p26-p11.1 (loss), 3q26.2-29 (gain) and 6q25.3-24.3 (loss) in individuals presenting with endobronchial squamous metaplasia. In that earlier study they were able to use the presence of these CNVs as predictors of cancer diagnosed within 44 months with 97% accuracy. In the current study they evaluated this CNV-based classifier with an independent set of 12 samples (10 men and 2 women). Each had a carcinoma in situ or invasive carcinoma at the same site at follow-up. The authors examined the endobronchial squamous metaplastic and dysplastic lesions of these 12 patients. They also selected 24 control subjects with dysplastic lesions and a similar distribution of baseline histopathology and clinical and demographic variables. They identified a number of CNVs in nine cases. The 24 controls and three cases contained only a few genomic alterations. Validation of their predefined CNV-based classifier demonstrated 92% prediction accuracy. The negative predictive value of this classifier was 89% and the authors were able to classify the 24 controls and three cases as low risk. Combining results from their previous study (18 cases and 47 controls), they were still able to confirm these three CNVs as parameters of the classifier. Interestingly, the gain at 3q26.2-q29 contributed the most to the classification, being present in virtually all lesions. The PIK3CA gene resides in this region and evaluation of the number of copies of this gene gave very similar results to those from aCGH.

The authors claimed that CNV detection could be performed on sputum or bronchial brushings. Given the relatively small number of samples examined, larger cohorts need to be examined to confirm this finding and to possibly refine the regions of CNV. This might indicate which critical genes in each interval are being subjected to CNV which themselves might become novel therapeutic targets. This type of approach paves the way for future molecular analyses to assist in selecting subjects with endobronchial squamous metaplastic or dysplastic lesions who might benefit from more aggressive therapeutic intervention or surveillance.

Competing interests None.

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