Minichromosome maintenance proteins in cancer screening

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Early detection of cancer leads to more effective treatment and improved patient survival. Many current cancer screening tests examine cells obtained from the surface of stratified epithelia, such as those of the cervix, urinary bladder, large bowel or airways. However, identification of malignant or pre-malignant cells in conventionally stained cytological preparations from these sites is difficult. Abnormal cells may show subtle morphological changes that are difficult to distinguish from benign reactive features, and are frequently missed, particularly if present in low numbers. Biomarkers capable of detecting abnormal cells with adequate clinical sensitivity and specificity would represent a significant translational advance, by providing improved diagnostic accuracy, reproducibility and the potential for automation, leading to clinical and economic benefits.

We consider minichromosome maintenance proteins 2–7 (MCMs) to be excellent markers suitable for detection of several different cancer types in cytological specimens. These proteins form a hetero-hexameric complex that is indispensable for DNA replication during the S phase of the cell cycle [1]. MCMs are not cancer specific proteins, being essential for replication of normal as well as neoplastic cells. Their value as cancer markers comes from differences in their micro-anatomical distribution. In normal stratified epithelia, MCMs are restricted to basal proliferative compartments, being rapidly lost from differentiating cells. As cells obtained in cytological preparations generally emanate from surface layers, either through passive shedding or active sampling by swabbing, brushing etc., the normal cells present in cytological samples are MCM negative. In contrast, malignancy and premalignancy are characterised by cell cycle deregulation, leading to abundant expression of MCM proteins in nuclei throughout the full thickness of stratified epithelia, including in surface layers [1]. Detection of MCM proteins in cytological preparations therefore enables neoplastic cells to be distinguished from their normal counterparts.

MCMs offer theoretical and practical advantages over other biomarkers of proliferating cells, such as Ki67 and proliferating cell nuclear antigen (PCNA) [2]. Ki67 is absent from a proportion of cells in each phase of the cell cycle, including the S phase, and has consistently been observed to be expressed in fewer malignant/pre-malignant cells than MCMs (particularly in surface layers), at a wide range of anatomical sites. PCNA is involved in DNA repair, thereby providing less specificity for cancer detection, and it shows striking fluctuations in abundance between cells, making interpretation of stained cytology samples very difficult. A number of alternative markers of cycling cells are emerging, although none has been demonstrated to show the strong clinical performance of the MCMs.

The presence of MCM stained cells can be detected using a variety of strategies, all of which have potential for automation. Our preferred method is immunocytochemistry. Abnormal cells stain with crisp nuclear signals, making them easy to identify, even at low magnification. This technique is particularly suitable for identifying abnormal cells that are present in low numbers, for example, using automated microscopy. Samples with rare abnormal cells are often falsely called negative by conventional cytological analysis [3]. MCM detection is particularly well suited to liquid based cytology (LBC) samples, which facilitate immunocytochemistry (particularly following ethanol-based fixation), make cell identification easier and permit multiplex testing. However, the technique can also be applied to non-LBC samples, for example, in limited-resource settings. We recently demonstrated that MCM staining of non-LBC cervical smears in India increased diagnostic accuracy and inter-observer agreement and made slide assessment easier and substantially faster [4].

An alternative strategy for detection of MCM positive cells is to use a liquid phase assay, such as DELFIA or ELISA. This approach has yielded encouraging data in settings where neoplastic cells, when present, are not substantially outnumbered by normal cells, most notably screening for transitional cell carcinoma using urine sediments [1]. In contrast, the sensitivity of liquid based assays is compromised

in sample types where abnormal cells are often present in a small minority (e.g. cervical smears, sputum), as the signal is diluted following cell lysis, rather than being retained specifically in the abnormal cells.

As with all screening tests, MCM detection is vulnerable to false results. False negative tests (reducing sensitivity) generally result from a failure to sample a lesion, while false positives (reducing specificity) can occur when cells at an epithelial surface enter the cell cycle for non-neoplastic reasons, for example, in wound healing or severe inflammation [5]. In our experience, the intensity of MCM staining in reactive/reparative conditions is less than that of neoplastic cells. For cervical smears (and in principle for other sample types too) it is possible to alter staining parameters to modulate analytical sensitivity for abnormal cells. 'Dialling down' analytical sensitivity results in fewer false positive reactions but does not affect overall clinical sensitivity for disease, as the number of immunostained cells in such samples remains high enough for the case to be called test positive.

Current work in our laboratories is focussed on generating algorithms for applying MCM immunocytochemistry in specific clinical settings, particularly in determining the most appropriate combination(s) with other screening approaches. For example, MCM testing has the potential to be paired with human papillomavirus testing in cervical smears [3], faecal occult blood testing in stools [6] and chest imaging in sputum samples. Whether these various combinations are best applied in parallel or series and, if the latter, in which order, remains to be determined. Nevertheless, the clinical potential of MCMs in improving early diagnosis of several common cancers continues to be supported by data emerging from our ongoing translational studies.

Conflict of interest statement

The authors are entitled to a share of royalties received by Cancer Research Technology Ltd on sales of products related to the use of MCM detection in cancer diagnosis.

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