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Rapid Communication

Molecular markers: How to apply in practice

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Rationale

Predicting disease outcome is a major challenge in modern medicine. Concerning the prediction of HPV-associated cervical disease, several important issues are related both to the management of women with diagnosed cervical precancer and cancer. The detailed molecular mechanisms explaining the different oncogenic potential of the LR-HPV and HR-HPV have emerged only recently. Oncogenic HPVs are capable of contributing to the development of the malignant phenotype by several different mechanisms, most of which seem to be closely interrelated [1]. Because of the fact that these molecular interactions are mediated by proteins, the logical strategy to dissect the complex molecular pathways is to study these molecular markers, using a variety of research techniques [1,2].

Biomarkers are measurable indicators of exposure effects and susceptibility or disease state, and are used to understand the mechanisms of cancer progression. Considering the trend of increase in incidence rate of the cervical cancer and its high prevalence in developing countries, there is a need to identify and validate new markers for cervical cancer detection. Apart from predicting disease outcome and viral events, effective and specific molecular markers would not be susceptible to the many technical limitations of current cervical cancer technologies that rely primarily on the morphological interpretation of cells sampled from the uterine cervix. The accuracy of screening based on morphological interpretation of cytological samples, can be substantially hampered by low inter-observer reproducibility [3]. Molecular markers are therefore perceived as having the potential to confer an improved level of reproducibility as compared to conventional screening tools. This document is focused on issues related to the use of markers as potential screening tools in early detection of cervical cancer precursors.

Evidence based approach to delineate molecular markers for cervical cancer

Based on the major pathogenetic events involved in cervical carcinogenesis, three different levels of risk may be differentiated in the development of cervical cancer and its precursor lesions:

- infection of the epithelium with oncogenic HR-HPV types,
- emergence of cell clones with deregulated expression of viral oncogenes in basal and parabasal cell layers (initiation), and
- progression of preneoplastic lesions to invasive carcinomas (progression).

Molecular marker-based assays can be designed and applied to delineate these three steps [3–5] and have the potential therefore to inform and/or replace current cancer management/screening systems. Accordingly, the potentially promising markers can be categorized into different classes such as chromosomal anomalies, DNA adduct formation, cell cycle check points, oncogene expression/function, tumor suppressor gene expression, apoptotic markers, epigenetic regulation such as methylation, metabolic markers and imaging markers [1,2]. The advantages of one class of a marker over others and their clinical implications in cervical cancer screening are under intense study at the moment.

The detection of HPV infections by the commercially available HPV tests serves as an excellent test to identify the patients at risk (level one); the majority are based on the presence or absence of the major structural gene: L1, for a pool of HR-HPV types. HR-HPV infections are required to induce transformation in most cases. However, since most HPV infections resolve spontaneously and only very few of the infected women finally develop clinically relevant lesions, one positive HPV test does not justify medical intervention in itself, but rather identifies women who have an elevated risk as long as the infection persists.

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The major pathogenic events that provoke transformation of the cervical epithelium are initiated by the deregulated, aberrant expression of the viral oncogenes E6 and E7 in replication competent basal and parabasal cells of the epithelium. During a normal viral life cycle, these genes are only expressed in terminally differentiated cells in the more superficial cell layers of the cervical epithelium, that are irreversibly cell cycle arrested. In these cells, viral oncogenes are unlikely to damage the cells. If, however, these genes are expressed at a high level in not yet terminally differentiated cells, they may abrogate normal regulation of the cell cycling and mitosis, thereby inducing chromosomal instability that ultimately results in neoplastic transformation of the respective cell clones. A feature of CIN2/3 lesions (compared with CIN1 lesions and normal cervix) is the up-regulation of E7 expression, which can be detected throughout the thickness of the epithelium.

The detection of cellular changes that go along with the deregulated expression of the viral oncogenes in basal and parabasal cells may therefore add very important diagnostic information. Those markers may indicate cell populations that have just reached the risk level 2. Others may rather reflect the consequences of chromosomal instability and therefore be better suited as progression markers (risk level 3, progression to invasive carcinomas).

Direction of further research

Several lines of research are currently been followed to identify appropriate markers:

- the detection of oncogenic viral gene activity by detecting transcripts of the E6 and E7 genes for example with the RNA proofer technology (NorChip),
- alterations of the methylation pattern of several cellular genes that may efficiently predict the onset of neoplastic transformation (initiation),
- changes of chromosomal or viral genomes (artificial intelligence, AI) methods in distinct genomic regions or changes of the structural integrity of the viral genomes (integration),
- identification of cellular proteins that are over-expressed by HPV infected cells in response to the expression of the viral oncogenes E and E7.

Recent work has suggested that there are a couple of cellular gene products that may either directly or indirectly be activated by the deregulated expression pattern of the viral oncogenes in the basal and parabasal cell compartment of the epithelium. Such cellular markers may have advantages above the viral markers since only one or few antibodies directed against the respective cellular proteins may confer the same diagnostic information, independent of the underlying activity of the specific HR-HPV genotype (or types) mediating the disease. The prototype marker of this category is the $p16^{INK4a}$ gene product that apparently confers the most precise diagnostic information to identify cells that display the deregulated gene expression profile to the HR-HPV oncogenes [3,4]. Other markers include the HSP 70, survivin, various proliferation markers including the mcm antigens, and DNA polymerase alpha, C4.8/NET-1, to name a few [1,2,6,7]. Future research that was intensively discussed during the EUROGIN 2006 Conference will reveal, whether these markers may add useful diagnostic information and what their role in forthcoming cervical cancer screening programs may be.

Clinical perspectives

The current status of the clinical evaluation of these concepts suggests that the majority of these markers tend to more likely point to progression of already initiated lesions and that the may lack sufficiently sensitivity to identify all initiated lesions, however there is clearly a substantial lack of sufficiently large data sets to finally conclude on their clinical diagnostic value [3,4]. Similarly, large, prospective cohort studies are needed, where conventional analysis of (histology-confirmed) disease outcomes from a baseline of normal are performed alongside marker detection/evaluation?

Markers are measurable indicators of exposure effects, susceptibility or disease state, and are used to elucidate the mechanisms of cancer progression. Ideally, a marker should be inexpensive and easy to assay in non-invasively collected fluid or cellular samples, have a high sensitivity and specificity, and utilize automatic high-throughput technology [1,2]. It is hoped that in the future such a marker will be developed. Regardless of the kind and the intended application of markers, there are fundamental analytical issues that need to be addressed: reliability, precision, accuracy, and validity. The variance of the detection tests and the intra- and inter-laboratory variances must be known. In addition, the marker must be a determinant of outcome and should be a marker that is a candidate for targeted (intervention or prevention) therapy. Modulation of the risk marker should also be associated with a decrease in cancer incidence. Molecular alterations generally contribute towards risk assessment. Identification of populations at risk of developing cervical cancer is important because it provides opportunities for prevention and treatment.

Recommendations

Mortality due to cervical cancer has declined in many of the Western countries over the past decades, largely due to advances in technology and increased compliance with cancer screening. This decline could also be attributed to improvements in early diagnosis of the disease by utilizing specific molecular markers. Until now, a number of potential markers have been identified which, after clinical validation should contribute in diagnosis, prognosis, and treatment of cervical cancer [1–4,6]. So far, detection of HPV (using different assays) has been the most promising clinical application [1,5,8]. In cervical cancer research, well-characterized clinical materials including both precancer and cancer lesions enable the use of different outcome measures as dependent variables in univariate and multivariate analysis, to disclose the potential

predictive factors of these outcomes of interest (disease outcome and viral events) [1,6,8]. Apart from getting new insights in the molecular pathogenesis of HPV-associated cervical carcinogenesis, we anticipate to disclose individual markers, a set of markers, or an expression profile of any such marker sets that would be of clinical value as predictors of disease outcome in cervical carcinogenesis and preceding HR-HPV infection [2].

In cervical cancer screening, the use of multiple markers that can complement each other for better assessment remains the current trend among the investigators in this area. Such combination will also remarkably increase the sensitivity and specificity of the assay [3,4]. Based on these well-characterized molecular markers, highly accurate screening for cervical cancer and CIN should be technically feasible in the near future. Considering the rapid advancement in the modern technology for detecting cancer precursors, it is not a conceptual or technical challenge, but a matter of health care and financing which delay the elimination of this malignancy on the global scale.

Appendix A

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