Cancer-Risk-Predictive Epigenetic Markers for Oral Premalignant Lesions

Subjects: Oncology

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Epigenetic regulation has emerged as a mechanism of intense research interest that captures the early impact of environmental insults on the genome and may provide key information on the progression of complex diseases, such as cancers, metabolic disorders, cardiovascular diseases, and neurodegenerative diseases. The keywords used to identify studies on epigenetic oral cancer risk-predictive markers include: (1) primary samples from pre-malignant dysplasia and/or leukoplakia of any grade (low-grade, high-grade, mild, moderate, or severe) collected before they either advanced to cancer (progressive OPLs) or remained OPLs (static OPLs); and (2) samples with longitudinally followed outcomes. Besides these two inclusion criteria, additional search terms used for identifying studies on epigenetic risk-predictive markers included risk-predictive, oral squamous cell carcinoma (OSCC), predictive biomarkers, pre-malignant oral lesions, epigenetic predictive biomarkers, epigenetic OSCC risk factors, epigenetic OSCC risk, epigenetic biomarkers oral dysplasia, methylation in oral dysplasia, methylation OSCC prediction, methylation OSCC risk, miRNA OSCC prediction, miRNA OSCC risk, and/or histone modification OSCC.

Keywords: DNA methylation ; epigenetic

1. Cancer risk-predictive epigenetic markers for OPLs

Epigenetic regulation has emerged as a mechanism of intense research interest that captures the early impact of environmental insults on the genome and may provide key information on the progression of complex diseases, such as cancers, metabolic disorders, cardiovascular diseases, and neurodegenerative diseases [1]. Search criteria for identifying studies on epigenetic risk-predictive markers include: (1) primary samples from pre-malignant dysplasia and/or leukoplakia of any grade (low-grade, high-grade, mild, moderate, or severe) collected before they either advanced to cancer (progressive OPLs) or remained OPLs (static OPLs); (2) samples with longitudinally followed outcomes; and (3) search terms such as risk-predictive, OSCC, predictive biomarkers, pre-malignant oral lesions, epigenetic predictive biomarkers, epigenetic OSCC risk factors, epigenetic OSCC risk, epigenetic biomarkers oral dysplasia, methylation in oral dysplasia, methylation OSCC prediction, methylation OSCC risk, miRNA OSCC prediction, miRNA OSCC risk, and/or histone modification OSCC.

1.1 DNA methylation

Aberrant methylation has been observed as an early molecular event in oral carcinogenesis and, therefore, may serve as a risk-assessment marker for cancer prediction and prevention [2][3]. Most published studies on DNA methylation in oral carcinogenesis focused on analyzing samples of different lesion types collected from different patients (cross-sectional), which provide little predictive value in evaluating the future outcome of OPLs. Some studies analyzed dysplastic samples with different follow-up outcomes but used gene-specific approaches, which did not allow for the discovery of new targets.

The gene-specific approaches used to identify potential risk-predictive markers in OPLs most frequently involve promoter methylation of tumor suppressor genes. Inactivation of $p16^{INK4a}$ via CpG methylation has been described as a key event in epithelial dysplasia, with multiple longitudinal-model studies suggesting its role in malignant conversion and risk prediction. One study found increased hypermethylation of $p16^{INK4a}$ (57.7%) and $p14^{ARF}$ (3.8%) as well as mutations and deletions in those genes in oral dysplastic lesions [4]. $p16^{INK4a}$ hypermethylation was also associated with LOH on two or more of the following three markers: IFNα, D9S1748, and D9S171. However, follow-up information was not yet available. Therefore, the significance of those DNA methylation changes in predicting cancer progression remains to be determined. Four studies explored the DNA methylation patterns of $p16^{INK4a}$ in longitudinally followed oral dysplasia and observed increased DNA methylation of $p16^{INK4a}$ in progressive compared to non-progressive dysplasia [5][6][7][8]. The following three studies utilized the same patient cohort for prospective study. Cao et al. identified an association between $p16^{INK4a}$ hypermethylation and malignant transformation with 63.6% sensitivity and 67.9% specificity [8], whereas Liu et al.

found an association with 62% sensitivity and 76% specificity [6][7]. Together, these studies suggest that p16 methylation is a frequent event preceding cancer development in OPLs and serves as a cancer-risk-predictive marker for OPLs.

To date, only seven published studies have investigated oral carcinogenesis (not HNSCC) using genome-wide approaches [9][10][11][12][13][14][15]. All seven studies used microarray-based platforms (e.g., Infinium Human Methylation 27K or 450K BeadChip or Agilent 4 × 44 CGH Microarray), which, unlike the whole-genome bisulfite sequencing (WGBS) approach, survey only a small percentage (up to ~2%) of the total epigenome. Five of the seven studies were based on a cross-sectional design, with three looking for DNA methylation differences between paired OSCCs and their adjacent normal tissues [9][10][11] and two looking for differences between non-paired OSCCs and normal tissue [12][15]. One of the seven studies analyzed the TCGA methylomic data using the Infinium Human Methylation 450 BeadChip to determine the DNA methylation differences between OSCCs with different survival outcomes (i.e., prognostic markers) [14]. Only one of the seven studies provided a genome-wide analysis of DNA methylation changes between 12 pairs of oral dysplastic samples with different follow-up outcomes, with progressive samples developing OSCCs in 2.15 years (mean) and non-progressive samples remaining dysplasias for 7.64 years (mean) [13]. Using the Agilent 4 × 44 CGH Microarray, which contained 44,674 probes that covered 8369 genes, Foy et al. identified hypermethylation on 86 genes and hypomethylation on Long Interspersed Elements 1 (LINE1) in patients who developed OSCC compared to those who did not. LINE1 is distributed widely across the genome, and its DNA methylation status can be used to indicate global DNA methylation. Most of the 86 hypermethylated genes were also found to be downregulated and their promoter regions were hypermethylated in OSCCs compared to normal tissue. Hypermethylation on the promoter regions of angiotensin II receptor type 1 (AGTR1), forkhead box I2 (FOXI2), and proenkephalin (PENK) was further validated by pyrosequencing (p = 0.003). Overall, oral carcinogenesis appears to be associated with global hypomethylation on CpG islands and hypermethylation on the promoter regions of specific genes that presumably play a tumor suppression role.

1.2 miRNA and histone modification

Besides DNA methylation, the other two epigenetic mechanisms are microRNA (miRNA) expression and histone modification. miRNAs regulate gene expression at the post-transcriptional level by silencing mRNA targets. To date, there are only four longitudinally designed studies looking at the use of miRNAs in predicting the cancer risk of OPLs. No longitudinal study on histone modification in oral precancers has been found. The first miRNA study performed a genome-wide analysis on miRNA expression in leukoplakia with different five-year follow-up outcomes [16]. In this study, leukoplakia lesions included hyperplasia, hyperplasia with hyperkeratosis, and low-grade dysplasia. Following total RNA extraction and qRT-PCR sequencing, those with the most predictive potential were identified using the DESeq Bioconductor package. Four miRNAs, i.e., 208b-3p, 204-5p, 129-2-3p, and 3065-5p, were reported to show a relatively high sensitivity (76.9%) and specificity (73.7%) in differentiating high-risk vs. low-risk lesions. Based on the proposed model that combined the expression of these four miRNAs with age and histological information, 80% of the progressive cases and 63% of the non-progressive cases were correctly predicted. In the second study, global miRNA expression profiles were generated on progressive and non-progressive oral leukoplakia. The results showed that miR-21, miR-181b, miR-345, and miR-416a were found only in progressive leukoplakia and clustered OSCCs but not in non-progressive leukoplakia or normal tissue (p < 0.001) [17]. The third study by Hung et al. investigated the potential use of miR-21 and miR31 as markers to predict the progressive outcome of OPLs to OSCC, where OPLs included hyperkeratosis, hyperplasia, and dysplasia [18]. miR-31 was expressed more abundantly in progressive OPLs than in non-progressive OPLs, whereas miR-21 showed no difference between these two OPLs. The sensitivity and specificity for miR-31 were determined to be 87.51% and 73.3%, respectively. This finding indicates that miR-31 may be used as a marker to predict the progression of OPLs to OSCC. The fourth study by Harrandah et al. tested the expression levels of four miRNAs, i.e., miR-7, miR-21, miR-371, and miR-494, in progressive vs. non-progressive OPLs, defined as dysplasia, carcinoma in situ, and verrucous hyperplasia/verrucopapillary hyperkeratosis, in retrospective data with at least six months between dysplasia and malignant diagnosis. They reported that non-progressive OPLs expressed miR-375 at a higher level (p = 0.0004) but showed no difference in miR-7, miR-21, or miR-494 expression compared to progressive OPLs [19].

2. Challenges facing the discovery of predictive biomarkers

Several major barriers exist that limit progress in finding sensitive and reliable markers for predicting the risk of OPLs progression to OSCC. First, identification and validation of bona fide predictive markers requires either prospectively collected samples or archival samples collected before disease progression and individually followed for long-term outcome in the clinic. Both types of samples are exceedingly rare due to the cost- and time-consuming process of collection. Collecting samples for longitudinal studies with complete follow-up information has been challenging due to the required time, cost, and expertise, compounded by the loss of patient follow-up. As a result, population-based cohorts with long-term and comprehensive follow-up information and samples are either lacking or too small in sample size to achieve

the necessary analytical power, especially for whole-genome studies. Second, traditional markers based on protein expression are subject to pitfalls in the methods of detection. Third, the conventional candidate gene or pathway approach has a high likelihood of missing key players currently unknown to affect the disease of interest. Fourth, genetic mutations in OSCCs are wide-spread, irreversible, and sometimes confounded by secondary bystander events. Fifth, the power of genome-/epigenome-wide approaches is highly reliant on technical requirements, such as the quality of data and the analytical power of bioinformatic tools. Sixth, while whole-genome or genome-wide studies offer the advantage of comprehensive new discoveries, it is also difficult to pare down and authenticate all candidates identified by these approaches—a task further complicated by the limited availability of samples for validation. Lastly, although a longitudinal study design is the most ideal model for the discovery and validation of predictive markers, it is not without potential limitations. During the time of follow-up, patients may drop out of the study due to moving or death by other illness or may receive various treatments between sequential biopsies. Those treatments may certainly complicate the analysis by altering the expression of markers and/or the progression outcome of the lesion. This calls into question the significance and validity of markers obtained from samples where some patients received treatment and some did not. Another issue is that the length of time used by different studies to define progressive vs. non-progressive OPLs is somewhat arbitrary. Some studies did not provide sufficient information on follow-up time, and not all studies used the same criteria to define progressive vs. non-progressive cases.

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