MicroRNA in Cervical Lesions

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The regulatory functions of microRNA (miRNA) are involved in all processes contributing to carcinogenesis and response to viral infections. Cervical cancer in most cases is caused by the persistence of high-risk human papillomavirus (HR-HPV) infection. While oncogenic human papillomaviruses induce aberrant expression of many cellular miRNAs, this dysregulation could be harnessed as a marker in early diagnosis of HR-HPV infection, cervical squamous intraepithelial lesions, and cancer. In recent years, growing data indicate that miRNAs show specific patterns at various stages of cervical pathology, that gives hope for the development of non-invasive diagnostic tests that take into account the heterogeneity of tumor-related changes. Due to this heterogeneity resulting in difficult to predict clinical outcomes, precise molecular tools are needed to improve the diagnostic and therapeutic process.

Keywords: cervical cancer ; cervical neoplasia ; HPV ; microRNA biomarkers ; miRNA

1. Introduction

Cancer is a group of diseases that in the age of molecular biology development is regarded as the genome disease at the cellular level. It arises from one cell containing an initial set of mutations, and the accumulation of mutations over time with genome instability is common. This leads to intra-tumor genetic heterogeneity characteristic for all types of cancer [1]. The hallmarks of carcinogenesis include cell functional changes: the ability to produce growth factors and lack of sensitivity to their suppressors, inhibition of apoptosis, unlimited replication potential, angiogenesis, invasion, metastasis, changed stress response, metabolic rewiring, and immune modulation. The significant factor contributing to the development of the disease, especially for the cancer caused by infectious agents, is long-lasting inflammation[2][3][4].

Human papillomavirus (HPV) is the second infectious agent in terms of the frequency of inducing oncogenesis in the world[5], and remains a causative agent of cervical, vulvar, anal canal, penile, and head and neck malignancy [6][7]. Cervical cancer (CC) is the fourth most common cancer among women worldwide[8], and high-risk human papillomavirus (HR-HPV) DNA is detectable in over 90% of affected tissues [9]. CC most frequently originates from squamous epithelium (squamous cell carcinoma—SCC) or glandular tissue (adenocarcinoma—AdC). Regardless of the histological type, CC is often asymptomatic and predominantly diagnosed in advanced stage, despite of the occurrence of preceding stages (cervical intraepithelial neoplasia—CIN 1-3) and their long-term development. Late diagnosis limits the effectiveness of available therapeutic methods, including surgical treatment, and chemo- and radiotherapy. Metastatic CC remains incurable and is characterized by 16% 5-year survival rates[10].

CC is preceded by several consecutive cervical intraepithelial lesions that are contributed to HPV-induced persistent infection[11]. The key event associated with the progression to high-grade and tumorigenic changes is the integration of viral DNA into host cell genetic material. This leads to over-expression of viral oncogenes that affect genetic instability and uncontrolled cell cycle progression [12]. Host genetic factors, including alterations in levels of oncogenes and tumor suppressors as well as chromosome aberrations and mentioned lifestyle factors have additional influence on disease development[13][14].

Since the 1950s, the gold standard in the screening of cervical intraepithelial lesions was cytological examination (Pap smear). Due to low sensitivity of these assays, an HR-HPV based preventive system (or co-testing with cytology) has been introduced in developed countries[15]. It was proved to be more effective for early detection of AdC, the frequency of which demonstrates a growing trend in highly developed countries [16]. It was estimated that the proportion of AdCs in relation to SCCs increases from 10% up to 25% of CC cases and becomes more common in younger women[17][18].

In the last decade, research based on explaining the molecular basis of oncogenesis and its utility in diagnostics has been extensively provided. Intensive investigations into the progression of tumorigenic changes are focused on the non-coding regions of the human genome. The central dogma of molecular biology assumes that genetic information transfers from DNA to RNA and then from RNA to proteins in transcription and translation processes. Although 70% of the genome can

be transcribed into RNA, the protein-coding sequences represent at most 2% of the human genome[19]. It turned out that nonprotein-coding regions of the genome exhibit crucial regulatory functions involved in the cell and tissue homeostasis. They are transcribed into the non-coding RNAs (ncRNA) from exon, intron, or intergenic sequences and play a key role in the post-transcriptional regulation of gene expression[20]. The most widely studied ncRNAs are microRNAs (miRNA)—RNA molecules up to 25 nt length that strongly regulate the expression of the hundreds target genes at the post-transcriptional level. miRNA affects mRNA by complete or partial hybridizing the seed sequence to the 3′ end of the untranslated region (3′UTR) of the target mRNA. It results in degradation of mRNA or block of translation[21].

It is well documented that miRNAs play an important role in processes that correspond to all the hallmarks of cancer[22]. Well confirmed variability of miRNA expression in a wide range of tumors has revealed significant correlations with the risk of cancer development, advancement of disease, metastasis ability, and therapeutic response to chemo- and radiotherapy[23][24]. Furthermore, in the cases of cervical pathology, specific aberrations in miRNA levels are characteristic for each stage of neoplasia and cancer [25].

Commonly used primary screening assays (exfoliative cytology and HR-HPV) are insufficient to detection of viral persistency and present small predictive value, especially in the risk prognosis of CIN 3 progression to cancer. Furthermore, inconclusive results of screening tests are effective in overusing of invasive diagnostic methods including colposcopy-directed biopsy to perform histological classification of changes[26]. Therefore, it is necessary to develop more efficient solutions, search for new diagnostic approaches to detect precancerous stages, and assess prognostic factors in advanced intraepithelial lesions and cancer.

miRNA pattern is considered as a specific fingerprint of cellular condition and a promising tool with great capabilities in development of personalized medicine. Advances of individualized diagnostics is particularly important in the case of tumors, due to the high heterogeneity even within the same histological type. To date, there has not been an algorithm established that incorporates the alterations of miRNA expression to CC screening. This review is focused on the current state of knowledge about the diversity of potential applications of human miRNA patterns in modern diagnostics at various stages of cervical lesions related to HPV infection and its progression to cancer.

2. Role of MicroRNA in Cervical Lesions

Cervical cancer continues to be at the forefront of mortality among middle-aged women, as evidenced by cancer statistics [5][8]. As most cervical cancers, especially in developing countries, are detected at an advanced stage, exploiting the potential of miRNAs as prognostic factors and as indicators of treatment susceptibility is well-founded. An effective screening of women from risk groups also remains extremely important.

miRNA, despite the intensification of research on their functionality and clinical utility, are still relatively poorly understood. However, it is known that their dynamic expression changes are associated with key determinants of neoplastic diseases[27]. Their impact on series of cell signaling pathways related to tumorigenesis creates a wide research field towards their use as diagnostic and prognostic (pre)cancer biomarkers[28][29][30][31][32]. miRNAs are relatively easy to measure under laboratory conditions due to the much greater stability of the molecules compared to other RNA fractions[33]. However, the techniques of isolation, determination, normalization, and analysis of results require standardization in order to obtain repeatability and comparability of test results obtained in various research centers. This comparability is also not fully effective due to different study designs; different criteria for selecting the reference group; not taking into account other cancer risk factors; and the individual variability, which remains extremely difficult to reliably assess.

Expression levels of miRNAs at different stages of cervical pathology usually fluctuate continuously, or statistically significant changes appear only at an advanced stage of cancer progression[34]. Moreover, the variation ranges of the expression level may overlap to a large extent, which can be problematic in establishing clear cut-off values. The best confirmed differential miRNA species in cervical lesions noted in the most of reports are upregulated miR-21, miR92a (early continuous increasing), miR-9, miR-15b, miR-16, miR-20b, miR-141, and miR-155 (late expression) and downregulated miR-100, miR-195, miR-203, miR-375, miR-424 (continuous decreasing of expression), miR-34a, miR-99a, and miR-125 (reduced commonly in cervical cancer). Moreover, the demonstrated discrepancies in the dynamics of changes in miRNA expression may indicate earlier disturbances in the cell balance, at a time when cytological/histological changes are still elusive. Such a feature, if confirmed in future research, could represent a significant advantage in terms of high predictive value of potential biomarkers.

Variation of the expression of miRNA is multifactorial[22]. Therefore, the search for one miRNA as the perfect marker seems to be a rather limited approach. It seems more reasonable to create a miRNA signature that takes into account most of these factors, corresponding to the patient's clinical condition, which could be a specific fingerprint of cervical pathology[29][35]. As described in Liu et al., a pattern of six miRNA species (miR-20a, miR-92a, miR-141, miR-183, miR-210, miR-944) achieved great diagnostic performance in differentiating cervical (pre)cancerous lesions[32]. Therefore, combination of several miRNAs can significantly improve the diagnostic accuracy of the assays, and, with the establishment of clear criteria for the results' interpretation, provide an efficient way to discriminate between various precancerous conditions. Furthermore, miRNA profile can be determined from the same sample as cytology and HR-HPV test, namely, from exfoliated cells noninvasively collected on the LBC medium[36][37][32].

Consideration of predictive and prognostic factors seems to be a big challenge, as there are still not enough reports of extensive studies in the reference group of women with various cervical pathological conditions. However, researchers are getting closer to establishing the relationship between miRNA expression levels and sensitivity to conventional methods of cervical cancer treatment that requires confirmation of results received in CC cell lines.

In view of heterogeneity of CC molecular features, it should not be treated as a single disease, both in the diagnostic and therapeutic process, and requires a personalized approach. Introducing new diagnostic solutions for personalized medicine is challengeable and requires accurate knowledge about a cell's dysregulation at the molecular level.

MiRNA profiling may provide a detailed fingerprint of a cell's condition and, in reference to current reports, seems to have high potential to be the marker-determining multifactorial process with relation to cervical neoplasia and cancer development, having strong predictive and prognostic value. However, the satisfactory preliminary results of scientists in this matter requires confirmation in standardized, extensive clinical trials conducted in experienced research centers to develop clear determination algorithms and introduce a new, broad-spectrum and noninvasive biomarker into the clinical use.

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