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Review

Molecular targets of HPV oncoproteins: Potential biomarkers for cervical carcinogenesis

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ABSTRACT

Cervical cancer is the second most common cancer among women worldwide and is responsible for 275,000 deaths each year. Persistent infection with high-risk human papillomavirus (HR-HPV) is an essential factor for the development of cervical cancer. Although the process is not fully understood, molecular mechanisms caused by HPV infection are necessary for its development and reveal a large number of potential biomarkers for diagnosis and prognosis. These molecules are host genes and/or proteins, and cellular microRNAs involved in cell cycle regulation that result from disturbed expression of HR-HPV E5, E6 and E7 oncoproteins. One of the current challenges in medicine is to discover potent biomarkers that can correctly diagnose cervical premalignant lesions and standardize clinical management. Currently, studies are showing that some of these molecules are potential biomarkers of cervical carcinogenesis, and it is possible to carry out a more accurate diagnosis and provide more appropriate follow-up treatment for women with cervical dysplasia. In this paper, we review recent research studies on cell cycle molecules deregulated by HPV infections, as well as their potential use for cervical cancer screening.

Contents

1. Introduction . . . 92
2. HPV-induced oncogenesis . . . 92
3. The potential biomarkers of cervical carcinogenesis . . . 92
   3.1. Molecular targets of HR-HPV E6/E7 oncoproteins . . . 92
      3.1.1. p16 ^{INK4a} . . . 92
      3.1.2. Ki-67 . . . 93
      3.1.3. ProEx C . . . 93
   3.2. Molecular targets of HPV E5 oncoprotein . . . 93
      3.2.1. Cell surface receptors . . . 93
      3.2.2. p21$^{Waf1/Sdil/Cipl}$ e p27^{KIP1} . . . 93
      3.2.3. COX-2, VEGF e Cav-1 . . . 93
   3.3. Host microRNAs modulated by HPV E5, E6 and E7 oncoproteins . . . 94
4. The potential use of biomarkers in cervical cancer screening . . . 94
   4.1. p16^{INK4a} . . . 94
   4.2. Ki-67 . . . 95
   4.3. p16/Ki67 . . . 96
   4.4. ProEx . . . 96
   4.5. Cell surface receptors . . . 97
   4.6. p21$^{Waf1/Sdil/Cipl}$ e p27^{KIP1} . . . 97
   4.7. COX-2, VEGF e Cav-1 . . . 97
   4.8. miRNAs . . . 97
5. Other forms of biomarkers . . . 98
6. Concluding remarks . . . 98
Acknowledgements . . . 98
References . . . 98

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1. Introduction

Cervical cancer is one of the most common cancers affecting women worldwide, and is responsible for 529.000 new cases and 275.000 deaths each year [1–3]. Over 80% of these cases occur in developing countries, where prevention and control programs are ineffective or non-existent [3,4].

The etiology of cervical cancer is related to persistent infection by Human Papillomavirus (HPV) [5,6]. There are more than 120 HPV types that have been classified into high-risk (hrHPV) and low-risk (lrHPV), depending on their oncogenic potential. At least 15 HPV types associated with malignancy of both genital tract and non-genital tract have been categorized as high-risk types. Among these, HPV-16 and -18 genotypes are detected in more than 70% of all cases of cervical cancer [7,8].

The conventional Pap test constitutes the main strategy used in screening programs for diagnosis of cervical intraepithelial neoplasias (CIN) and control of cervical cancer. However, there are often failures in the proper interpretation of the pap smears caused by inconsistencies in the results of the cytopathologists [9,10]. Recently, some countries have adopted HPV testing along with the Pap tests to provide more information about cervical cancer screening [11,12]. However, HPV testing does not have sufficient specificity owing to the high rate of insignificant HPV infections [13] and viral infection by itself it is not enough to cause this type of cancer [11]. Other factors related to the host such as genetics and immunity may play a significant role in the persistence of infection and progression of cervical carcinogenesis [14].

Recently, studies have shown that some host molecules are potential biomarkers of cervical cancer and represent an attractive alternative or supplement to cytological testing and HPV detection. Several of these molecules involved in cell cycle regulation are altered by the presence of HPV oncoproteins [10,14]. Searches for molecular targets have characterized the expression profile of proteins and the coding genes which are involved in cervical carcinogenesis with the aim of improving the diagnosis and treatment of cervical cancer [15–19].

More recently, studies have focused on the role of microRNAs (miRNAs) in the development of cervical cancer [20–23]. It is predicted that miRNAs regulate up to 90% of human genes, which suggests that they may control almost all the cellular processes [24,25]. The miRNA expression profiles (miRNA signatures) can be used as valuable biomarkers since they are an aid to the diagnosis and prognosis of various types of cancer [26–30]. However, as a result of disagreements between researchers, it has not been possible to characterize a miRNA profile associated with the cervical cancer.

In this scenario, several candidate biomarkers have been tested to improve the diagnostic accuracy of cervical dysplasias. Moreover, a growing body of scientific researchers has revealed molecules and pathways that are modulated by HPV in cervical carcinogenesis which suggests there is a potential for new biomarkers. In the light of this, the aim of this article is to discuss the potential biomarkers that are targets of HPV oncoproteins and their evaluation for clinical use in cervical carcinogenesis.

2. HPV-induced oncogenesis

The cycle of HPV induces proliferation of basal and parabasal cells, which leads to epithelial hyperplasia or papillomatosis with diverse extensions. This oncogenicity is due to the activity of E5, E6, and E7 viral proteins [31,32]. These oncoproteins result in continuous cell proliferation and an inability to repair possible damage in their genetic material, and thus accumulate rearrangements, aneuploidies and mutations that can lead to the development of cancer [33–35]. A number of routes and proteins of normal cells are altered, such as those involved in the division and apoptotic processes [36]. For example the E7 protein binds to tumor-suppressor proteins of the pRB family, and degrades them for uncontrolled activation of E2F transcription factor that stimulates expression of genes involved in S phase of the cell cycle [37]. Furthermore, E7 interacts with p21 and p27, which are the important inhibitors of cyclin-dependent kinases (CDK). The main target of CDK proteins is the cyclin-CDK2 in human keratinocytes which in turn regulates the transition from G1 to S phase [38]. The E6 protein also interferes with the pro-apoptotic functions of proteins such as Bak, Bax, c-myc and p53; for the maintenance of the neoplastic phenotype [39].

The integration of HPV sequences into the host genome is considered to be a crucial event in cervical tumor progression. This step occurs through a cleavage in the viral DNA in the cleavage site of the E1/E2 genes [40] causing deletion of the E2 gene and adjacent regions of the E4, E5, and L2 genes [41]. Thus, the transcriptional repression exerted by E2 is annulled, resulting in E6 and E7 overexpression, and severe malignancy [42–44]. Other studies have identified different targets for the E6 and E7 oncoproteins, including the following: telomerase [45], centrosome duplication factors [46] and signaling proteins [47,48].

Although the HPV E6 and E7 proteins are of crucial importance for HPV transforming properties[49,50,36], the role of the E5 protein in the development of cervical cancer has been increasingly explored. The activities of this oncoprotein support tumor progression, particularly in the early stages of the disease, since the E5 gene is deleted after the viral DNA has been integrated into host genome [51]. There are many mechanisms in which E5 is included that involve various signaling pathways for cell proliferation, angiogenesis and apoptosis [52–54]. One of the most well-known E5-mediated tumorigenic activities is its interaction with the epidermal growth factor receptor (EGFR), which leads to cell proliferation [55].

Another recently-discovered mechanism associated with HPV oncogenesis is the modulation of host miRNAs [56–58]. HPV interference in miRNA expression is caused by the fact that more than 50% of miRNA genes are located in the fragile sites or integration sites of hrHPVs [56]. Integration can alter miRNA expression via deletion, amplification, or genomic rearrangement. However, some functional studies have revealed that aberrant profiles of some miRNAs are due to the activities of E5, E6 and E7 oncoproteins [59,25].

3. The potential biomarkers of cervical carcinogenesis

Recently, there has been an increase in the number of studies of the genes and/or proteins, and cellular miRNAs that are involved in cervical carcinogenesis with the aim of finding an efficient biomarker as an alternative or supplement for cyto-histological tests and HPV detection [10,26,60]. Most of the suggested biomarkers are involved in cell cycle regulation disturbed by the expression of hrHPV E5, E6 and E7 oncoproteins (Fig. 1) [25,59,61].

Some of these potential biomarkers have been shown to be essential for immortalization of human genital keratinocytes and are involved in p53 and pRb pathways that are dysregulated by HPV E6 and E7 oncoproteins [50,62,63]. With regard to molecular targets of HPV E5 and their potential use for biomarkers, they are still in an early testing stage and need to be more fully explored. Studies have demonstrated that the E5 oncoprotein has the ability to induce tumors in transgenic mouse models, especially in the early stages of cervical carcinogenesis [64–67]. However, E5 also seems to play a role in the later stages of cervical carcinogenesis because it has been found to be expressed by episomal viral genomes that coexist with the integrated viral genomes in 26 to 76% of cases of cervical cancer [68–71].

3.1. Molecular targets of HR-HPV E6/E7 oncoproteins

3.1.1. p16 ^{INK4a}

The p16INK4a is a tumor suppressor protein encoded by CDKN2A gene that slows down the progression of the cell cycle by inactivation of cyclin D-CDK4/6 complex, which in turn promotes pRb phosphorylation for cell cycle progression [72]. In carcinogenic HPV-infected cells,

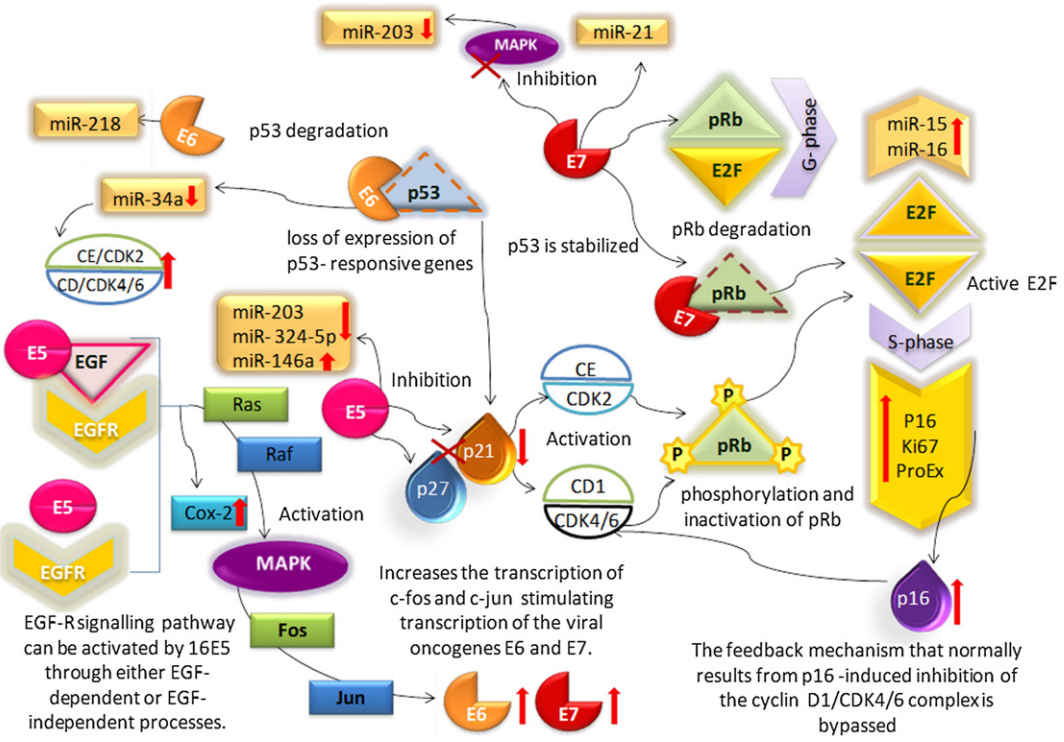

Fig. 1. Modulation of cell cycle molecules by HPV oncoproteins. HPV E7 binds to pRb and causes its degradation, leading to the displacement of active E2F and hence the activation of the S-phase genes (i.e. p16, Ki67, ProEx) in both the basal and parabasal cell layers. HPV E6 binds to p53 and targets its degradation which results in loss of expression of p53-responsive genes (i.e. p21) and HPV E5 active EGFR signaling pathway through either EGF-dependent or EGF-independent processes. Some miRNAs are also upregulated or downregulated by mechanisms of HPV oncoproteins in the host cell.

HPV E7 oncoprotein stimulates cell cycle progression to S phase through inactivation of pRb, and E2F transcription factor release, resulting in overexpression of p16INK4a [63,73,74]. Upregulation of p16INK4a induced by HPV E7 makes this protein a promising biomarker for cervical carcinogenesis [75–77].

3.1.2. Ki-67

The Ki-67 antigen is a nuclear protein associated with cell proliferation, that is expressed in all phases of the cell cycle (G1, S, G2/M) and encoded by the MKI67 gene [78]. Some authors have demonstrated increased expression of Ki-67 in the cervical epithelium due to the release of E2F transcription factor (HPV E7-mediated) [10,79,80]. One study correlated overexpression of Ki-67 with increased expression of cyclins D and E and the reduced expression of p21 and p27 tumor suppressors [10]. Ki-67 is considered to be a proliferation marker in basal cells, but also in intermediate and superficial squamous cells, since it shows association with different CIN grades and HPV infection [10,81].

3.1.3. ProEx C

The ProEx C includes topoisomerase II protein (TOP2) and the minichromosome maintenance complex II (MCM2) proteins. TOP2 is an enzyme which plays an important role in DNA replication and packaging [10,82,83]. MCM2 is a protein involved in the formation of replication forks and in the recruitment of other DNA replication related proteins. Both proteins play a significant role during the S phase of the cell cycle and are overexpressed in HPV-infected cells as a result of the uncontrolled activation of the gene transcription and aberrant induction of S phase in these cells [10,83].

3.2. Molecular targets of HPV E5 oncoprotein

3.2.1. Cell surface receptors

The interaction of HPV E5 protein with the epidermal growth factor receptor (EGFR) is one of the best known mechanisms that employ this viral oncoprotein. EGFR is a cell surface receptor tyrosine kinase for epidermal growth factor (EGF) with tyrosine kinase activity, and can be found in the epithelial cells of the cervix, including the mucosal cells [55,84,85]. E5 protein binds directly to the vacuolar ATPase of endosome implicated in the receptor degradation and inhibits its activity. This results in increased recycling of EGFR to the cell surface and enhanced receptor signaling [86,51]. Additionally, E5 exacerbates EGRF activity by increasing the phosphorylation of EGF [87]. These E5-mediated mechanisms activate signaling pathways such as Ras-Raf-MAP kinase and PI3K-Akt, which are involved in cell proliferation, angiogenesis and apoptosis. HPV16 E5 protein may also stimulate cell proliferation by activating G-protein-coupled receptors such as ETAR which promotes mitogenesis, angiogenesis and the cell invasion [52,53,88]. It has been seen that the presence of E5 in growth factor-starved keratinocytes increases the mitogenic activity of the ETAR ligand, endothelin-1 (ET-1), and causes high proliferation of E5-transfected cells, when compared with untransfected cells [89].

3.2.2. $p21^{Wafl/Sdil/Cipl}$ e $p27^{KIP1}$

Tumor suppressor proteins $p21^{Wafl/Sdil/Cipl}$ e $p27^{KIP1}$ are inhibitors of cyclin-dependent kinase (cyclin-dependent protein kinase inhibitors CKIs), and induce cell cycle arrest [54,90]. However, when regulated by HPV E5, these proteins are inhibited and cell cycle progression is continuously induced. Studies with immortalized human keratinocytes have detected suppression of p21 $^{Wafl/Sdil/Cipl}$ at the transcriptional level by E5 [90]. In contrast, HPV E5 downregulated the half-life of $p27^{KIP1}$ protein [54]. The effect of E5 on p27 ^{KIP1} is increased by activation of EGRF [89].

3.2.3. COX-2, VEGF e Cav-1

Cyclooxygenase-2 (COX-2), vascular endothelial growth factor (VEGF) and caveolin-1 (Cav-1) are cellular proteins upregulated by HPV E5. COX-2 is an enzyme which catalyzes conversion of arachidonic acid to prostaglandins and other eicosanoids, causing various cellular

responses such as cell cycle regulation, inhibition of apoptosis, extracellular matrix deposition and angiogenesis [91,92]. Thus, COX-2 plays a role in various types of cancer through the different stages of carcinogenesis [93–95].

VEGF is a member of the cytokines family that plays critical roles in physiological and pathological angiogenesis, and lymphangiogenesis [96]. Both proteins, COX-2 and VEGF, are induced by EGFR signaling pathway which is activated by the E5 oncoprotein [97,98]. As a result, the E5 oncoprotein also induces angiogenesis in cervical carcinogenesis, which is an essential stage in tumor growth and metastasis [99].

Caveolin-1 (Cav-1) is a membrane protein involved in cell differentiation and proliferation [100,101]. The E5-mediated upregulation of Cav-1 is not completely understood; however, this host protein may be involved in cell transformation by altering the EGFR signaling [102,103].

3.3. Host microRNAs modulated by HPV E5, E6 and E7 oncoproteins

MicroRNAs are endogenous non-coding RNAs of approximately 21 nucleotides in length that regulate gene expression by causing degradation or suppressing target messenger RNA (mRNA) translation [104,105]. The miRNAs have emerged in several current research studies due to their ability to regulate genes involved in important cellular processes such as proliferation, apoptosis and differentiation [106,107].

Some studies have evaluated persistent infection by hrHPVs by linking it with dysregulation of miRNAs in cervical cancers [57,108–110]. The aberrant expression profile of some miRNAs has been correlated with the activity of E6 and E7 oncoproteins [25]. For instance, downregulation of miR-218 [111,112] and miR-34a can be related to HPV E6 effects in the host cell [22,113]. The transcription of miR-34a is activated by p53, but this suppressor protein is degraded in cervical cancer cells by E6 oncoprotein, and thus reduces the expression levels of miR-34a [114,115]. Thus, miR-34a targets such as cyclin E2 and cyclin D1, CDK4, CDK6 among others, are overexpressed, and lead to cellular transformation [116,117].

Recently, Lin and Yao [118] have shown that increased expression of miR-21 induces proliferation, migration and apoptosis via CCL20 inhibition, a gene that is also targeted by E6 and E7 in HPV16-positive cervical cells. Melar-New and Laimins [119], in turn, have shown that HPV E7 downregulates miR-203 through the inhibition of the MAPK pathway/PKC that activates transcription of this miRNA. This mechanism results in increased expression of ΔNp63, a target of miR-203 which is responsible for the proliferation of more differentiated keratinocytes. Other miRNAs such as miR-15a and miR-16 are also modulated by HPV E7. Overexpression of these miRNAs is a direct consequence of E7-mediated inactivation of the pRB-E2F control mechanism that triggers continuous transcription of these miRNAs [25].

Finally, Greco and colleagues [59] suggested that modulation of miRNAs might also be related to E5 oncoprotein, which is most active in the early stages of cervical carcinogenesis [70]. This study showed downregulation of miR-203 and miR-324-5, and upregulation of miR-146a in HPV16-positive keratinocytes expressing E5. However other studies with clinical samples should be conducted to confirm the significance of both the E6 and E7, and E5 oncoproteins of miRNA dysregulation in cervical neoplasias.

4. The potential use of biomarkers in cervical cancer screening

The Pap test (cervical cytology test) is the standard method used to screen low-grade squamous intraepithelial lesions (LSIL) and high-grade squamous intraepithelial lesions (HSIL), which are also classified as cervical intraepithelial neoplasia (CIN) 1, 2, and 3. Despite its high specificity, the cytology test is relatively insensitive due to its poor inter-observer reproducibility and requires frequent sampling [120–123]. The discovery of HPV and the fact that it was an essentail factor in the development of cervical cancer, was a great advance. Studies show that the hrHPV test has a sensitivity of over 90% in the detection of CIN2 and CIN3 [124,125] compared to the cytological test; however, this is insufficient specificity due to the high rate of insignificant HPV infections [13]. Thus, the value of hrHPV DNA testing as a stand-alone cervical screening test compared to cytology is still open to question [10,12,28,126,27]. Nevertheless, the incorporation of HPV testing as an adjunct to Pap cytology in clinical practice, has improved the diagnosis of precancerous lesions and has been used by some developed countries in Europe and North America, especially in triage of women with atypical squamous cells of undetermined significance (ASC-US) on cytology and in women over 30 years old [12,127,128]. However, HPV tests tend to be expensive and are thus not readily available to all who might benefit from them, as in developing countries where more than 80% of cases occur [12,129–131]. Hence, there is a strong demand for additional, more sensitive, and specific biomarkers to improve the screening of women with cervical dysplasia. The interplay between HPV and host cell molecules has revealed a large number of these potential biomarkers (Fig. 2).

4.1. p16^{INK4a}

The p16 is proving to be a promising biomarker of carcinogenesis in various ways. The relationship between increased overexpression of p16 ^{INK4a} and the severity of the lesion is already being strengthened, both in the CINs of the histology and the HSIL of the cytology (Fig. 2a). This relationship reflects a single process that has already triggered the malignant transformation of the epithelial cells of the uterine cervix ([132–137].

The p16^{INK4a} has demonstrated a capacity to detect CINs or SILs in triage of women with equivocal cyto-histological results, including ASC-US, LSIL and atypical squamous cells-cannot exclude high-grade squamous intraepithelial lesions (ASC-H). As a result, the improved diagnostic accuracy of cervical cancer by p16INK4a detection has been evaluated as a complementary test to the Pap test, or even as a substitute for current HPV testing. In a recent study, in which a meta-analysis was conducted by Roelens and coworkers, [138] it was found that p16^{INK4a} had a greater degree of accuracy with regard to the HC2 test (Hybrid Capture 2) for the detection of CIN2 + in the triage screening of women with ASC-US. This was due to a greater specificity of p16^{INK4a}, which can prevent overtreatment of healthy women, without losing sensitivity (Fig. 2b). The p16^{INK4a} has already shown a greater specificity than HC2 for the triage screening of LSIL (Fig. 2c), although with less sensitivity it can result in undertreatment of sick women. As well as confirming the importance of the use of p16^{INK4a} for the detection of CIN2 + in the ASC and SIL after screening with hrHPV, Gustinucci and coworkers [139] still stressed its potential as a biomarker for CIN2 +, especially in cases of cyto-histological discordance. Although, in this study, it was found that 8 cases were histologically negative or CIN1 in the first biopsy, (diagnosed as p16 + ASC-H and HSIL-CIN3), there were in fact four cases of CIN2 and 4 of CIN 3. The p16^{INK4a} has also shown it can be correlated with the aggravation of cervical lesions in an independent way from hrHPV [140,141].

Some authors have raised the possibility of p16^{INK4a} establishment as a marker to replace hrHPV although they state that its clinical use is still limited by the lack of a standardized immunohistologic and cytologic scoring system [142]. However, some studies have determined that a combination of the detection of hrHPV with p16^{INK4a} results in a greater degree of diagnostic accuracy, as well as being able to provide more detailed information about the risk of the malignant transformation of squamous epithelial cervical lesions than the mere detection of hrHPV. This is because it has already been confirmed that the overexpression of p16^{INK4a} results in the inactivation of pRB by the E7 viral oncoprotein, which suggests that the viral DNA integration into host genome as well as tumorigenesis, have already been triggered. Additionally, the studies have raised the development of a simple and low-cost technology, based on p16INK4a biomarker, for the study of patients infected

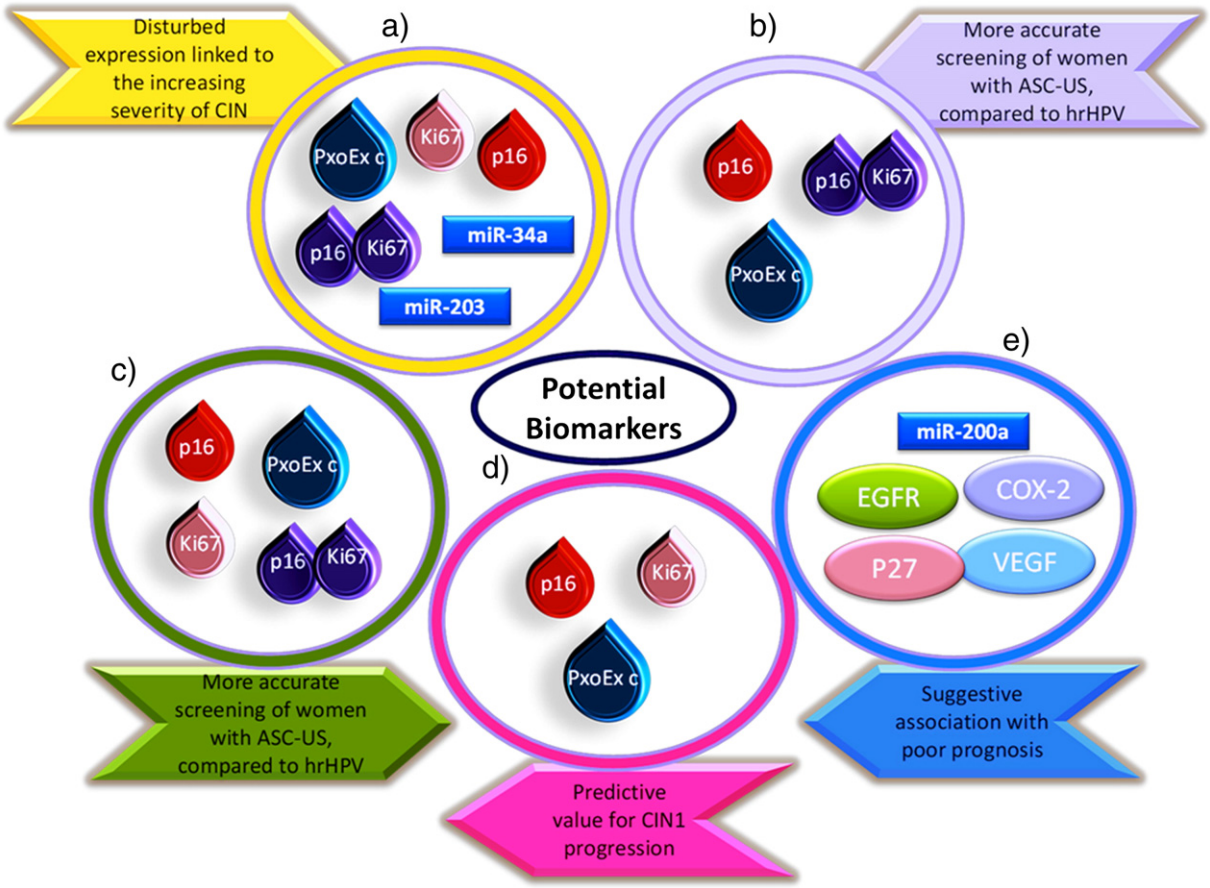

Fig. 2. Potential biomarkers of cervical cancer. Schematic representation of some molecular targets modulated by HPV E5, E6 and E7 oncoproteins, cited as potential biomarkers of cervical cancer.

with the hrHPV and diagnosed with LSIL of uncertain behavior [141,143–145].

The expression of gene CDKN2A that encodes the $p16^{INK4a}$ is also being investigated by qPCR or a microarray. Boulet and coworkers [146] showed that there is no correlation between the expression of the gene that encodes the $p16^{INK4a}$ and the cytological diagnosis of the HPV. Nonetheless, they show that this might be the result of the dilution of the mRNAs of the dysplastic cells by the mRNAs of the normal cells, which makes the detection of their overexpression by RT-PCR difficult. In contrast, more recent studies of mRNA expression have supported the results of studies of the immunostaining of $p16^{INK4a}$ and stressed the role of $p16^{INK4a}$ as a potential biomarker of the HSIL [135,147]. It should be pointed out that the differences between these studies might be due to the different platforms used for the evaluation of the mRNAs.

The $p16^{INK4a}$ is also being assessed as a predictor of the progression or regression of initial cervical lesions. In the study of Huang and coworkers [133], a difference of expression of $p16^{INK4a}$ was found between the CIN1 and CIN2/3 groups which shows that whether individually or in combination with the detection of hrHPV, $p16^{INK4a}$ improves the prediction of the progression of initial cervical lesions by identifying patients with CIN1 with greater precision (Fig. 2d) and thus allows careful monitoring and early treatment of these patients. In a meta-analysis, Tosoumpou and coworkers, [132] provided some evidence that the $p16^{INK4a}$ in histological samples could be a predictor of the progression of the disease [84,51,106,109] by identifying low-grade lesions (CIN1) which need closer monitoring. In a study of Nishio and coworkers [136], overexpression of $p16^{INK4a}$ was correlated with the diagnosis of CIN1-2 and $p16^{INK4a}$ was suggested as a superior biomarker to foresee the results of CIN1-2 in comparison with the hrHPV genotyping; although the authors draw attention to the various aspects of this result. For this reason, some authors have found a correlation between the overexpression of $p16^{INK4a}$ and the risk of progression or regression of CINs both of CIN2 [148] and CIN1 to CIN3 [149,150]. However, in the study of Quint and coworkers [143] a greater risk of progression or the persistence of LSIL was found, not as a result of the overexpression of $p16^{INK4a}$ but of age, since a greater risk was found in patients over the age of 30. In addition, it was determined that it was the genotyping of HPV and not the markers of the cell cycle, including $p16^{INK4a}$ that can assist in the prognosis of the course of LSIL. According to Izadi-Mood [151], although a $p16^{INK4a}$ negative expression cannot definitively exclude the patient with a cervical lesion from the high risk category, the immunostaining test for $p16^{INK4a}$ can be used as a supplementary test for the early diagnosis of cervical cancer. The administration of the results of the CINs still represents a dilemma for gynecologists (particularly of CIN1) and this sometimes leads to the over- or undertreatment of the patients. Although immunostaining of $p16^{INK4a}$ requires uniformity in the scoring system, its use in the triage screening of high risk patients, especially among young patients, can assist in reducing the progression of precancerous cervical lesions in the near future.

4.2. Ki-67

The Ki-67 is also being investigated as a biomarker of cervical carcinogenesis, as it is an important indicator of cell proliferation [152,80]. Some studies have found a link between the expression of Ki-67 and the CIN grades (Fig. 2a) [153–157]. The use of Ki-67 as an adjunct test to increase the diagnostic precision of cervical lesions, including doubtful cases, is currently being assessed, either together with the hrHPV test or not. Mimica and coworkers [158] confirmed that the Ki-67

immunodetection could predict hrHPV and that the overexpression of this protein was related with severity of the lesion, with a greater expression of this protein in CIN2/CIN3, which supports its value as a more accurate auxiliary method for the classification of the CIN. Kruse and coworkers [159] confirmed that there was a high correlation between the Ki-67 expression and the presence of hrHPV, which suggests that they can be used to correct the diagnosis of uncertain cases. Similarly, Pirog and coworkers [160] estimated the accuracy of Ki-67 as an adjunct test in the cervical biopsy diagnosis (originally classified as normal and LSIL by cytopathological diagnosis) compared to the HPV test, and it was found that the ki67 had a higher sensitivity and specificity, which shows a highly sensitive and specific marker for the identification of LSIL (Fig. 2c), even when determining the diagnosis of uncertain cases.

The Ki-67 was also suggested to be employed together with the set of procedures for cervical cancer screening, as an adjunct to liquid-based cytology to identify HSIL and as a surrogate marker of HPV-16 infection [161]. The role of Ki-67 as a predictor of the progression or regression of cervical lesions is also being assessed. Addictionally, a study [162] proved that Ki-67 has a strong, independent prognostic value for progression, owing to the fact that there is a greater predictive value for progression in the precancerous low-grade lesions (Fig. 2d) than the histopathological classification criteria or the presence of the hrHPV. Similarly, other studies carried out by Kruse and coworkers [163] have confirmed that the prognostic value of the model for the risk of progression of KI-67, exceeds the value of the histopathological test [162,163].

4.3. p16/Ki67

Owing to the proven accuracy of $p16^{INK4a}$ and Ki-67 in the triage screening of cervical cancer, several studies carried out assessments to find out whether the two junction markers (p16/ki67) can achieve a greater degree of accuracy in the screening of cervical cancer. In addition, a commercial kit (the CINtecPLUS Test) is already being evaluated in several studies. Some of these that have assessed the diagnostic value of the CINtecPLUS Test for p16/Ki67, both for the CINs and the LSIL, have found a significant increase in the positivity Index for p16/ki-67 with severity of the lesion both for the cytological and histological alterations (Fig. 2a) and with a high association with CIN2 + [123,164].

The performance of p16/Ki-67 in cervical screening programs for women with premalignant lesions, including ASC-US and LSIL in HPV-positive, is being evaluated and seeks a uniform reduction of patient referrals to colposcopy. In the study of Wentzensen and coworkers [123], as well as having a similar sensitivity and a significant improvement in specificity for the detection of CIN2 + in triage of ASC-US and LSIL (Fig. B and c) with regard to the hr-HPV test; the p16/Ki-67 test showed that there was a possibility of reducing almost half the number of colposcopy patient referrals, compared with the current practice of screening for all hrHPV-positive, ASC-US and LSIL patients. Other studies that have also evaluated p16/Ki-67 CINtecPLUS show a similar sensitivity but a greater specificity with regard to the rhHPV test for the detection of ASC-US and LSIL [165–167].

As well as establishing a close link between p16/Ki-67 and hrHPV infection, Dona and coworkers [164] highlighted the fact that the association between p16/Ki-67 and HPV16/18 was twice as high as that of other types of hrHPV. Moreover the study of Rokita and coworkers [168] only assessed the diagnostic value of the p16/ki67 test with regard to the Pap test and confirmed a higher degree of accuracy for p16/Ki-67 in the recognition of cervical lesions from a cytological examination, including that for the screening of patients with ASC-US and LSIL. These studies confirm the accuracy of the p16/Ki-67 test and underline their importance in HPV screening programs to prevent cervical cancer. Other studies that did not employ the p16/Ki-67 test (CINtecPLUS) also confirm that these markers can be used as adjuncts to increase the sensitivity of cytological screening, as well as the specificity of the HPV assay [169–171]. However, it should be noted that there is a need for clear methodological norms for improving the dual immunodetection of these in a clinical environment. For this reason, Gertych and coworkers [171], employed an automated technique for evaluating the dual p16/Ki67 nuclear immunoreactivity in liquid-based Pap tests. The system comprises digitized images of stained smears for the analysis of p16/Ki-67 immunoreactivity in cervical cell nuclei, by means of algorithms developed by the authors. Features of the core immunoreactions were classified into four types of immunoreactivity: p16 positive (p16(+)/Ki67(−)), Ki67 positive (p16(−)/Ki67(+)), dual p16/Ki67 positive (p16(+)/Ki67(+)) and negative (p16(−)/Ki67(−)), respectively: The results of this method were correlated with readings conducted by two cytopathologists and showed high accuracy, sensitivity and specificity for the classification of p16 +/+ Ki67 positive nuclei. This suggests that this quantitative characterization of nuclear immunoreactivity was satisfactory with regard to the effectiveness and results of Pap smear screening.

4.4. ProEx

The ProEx (immunostaining of MCM2 and top2 proteins) is another promising biomarker which has been linked to the severity of cervical lesions (Fig. 2a) [156] and the presence of hrHPV [172]. Depuydt and coworkers [173] evaluated screening strategies for cervical cancer and put forward, as a better strategy, the first triage screening of hrHPV followed by immunostaining with ProEx. The ProEx also proved to be a promising marker for the confirmation of HSIL owing to a close link with this type of lesion [10,172,174,175] and also to identify HSIL mainly in triage of women with ASCUS and LSIL (Fig. 2b e c) [156,172]. As well as being useful in the identification of cervical lesions that are more likely to progress [176].

A recent study Alaghehbandan et al., [177] evaluated the clinical usefulness of the ProEx C (Becton Dickinson) and PreTect HPV-Proofer E6/E7 mRNA tests for the triage of ASCUS and LSIL cytology when compared with the Hybrid Capture 2 HPV DNA test (HC2) and stated that both tests have a similar performance with a higher specificity than HC2. However, these tests had less sensitivity than HC2 for the detection of CIN2 +. In this way, the authors made clear that although the PreTect HPV-Proofer and ProEx C can reduce the colposcopy referrals, they are going to lose a proportion of the CIN2 + cases. This is a serious limitation which must be taken into account in the screening of ASCUS and LSIL. In contrast, in a previous study, Siddiqui [178] showed that ProEx C led to an increase of both sensitivity and specificity, in comparison with HC2, for the detection of CIN2 + in triage of ASC-US cases. This study also showed a higher degree of sensitivity and specificity through Proex C test for the detection of CIN1 + in the cytological results of ASC-US. These studies found that ProEx C could be used to improve the diagnostic accuracy of cervical cytology and could also be useful as a cytological adjunct to improve the sensitivity and specificity of the Pap test. The role of ProEx C to predict the risk of progression of CIN1 (Fig. 2d) was evaluated in the study of Ozaki and coworkers [150]. This marker had an equally high sensitivity and a greater specificity to predict the progression of CIN2, than HC2. Aximu and coworkers [179] found that ProEx is also a potential adjunct tool in the histopathological diagnosis of cervical adenocarcinoma in situ (AIS) and invasive adenocarcinoma (AC), especially in difficult cases with small biopsies or foci of disease.

Some studies have compared ProEx with other biomarkers. For example, a study [149] found that p16, Ki-67 and ProEx, can all be useful for the diagnosis of lesions of the uterine cervix by improving diagnostic precision. Other studies found that ProEx is more selective and informative about the progression of LSIL than Ki-67 in liquid-based cytology and is also regarded as a better method for the detection of CIN3 compared with $p16^{INK4a}$ and Ki-67 [17,180]. In a more recent study [181] a platform was developed for the simultaneous and automated detection of ProExC/Ki-67 in cervical dysplastic cells in Liquid-based cytology for improved cervical cancer screening. According to the authors, a platform for high-content images could be used to evaluate and validate

new biomarkers or combinations of these, but stressed the fact that the evaluation and validation of biomarkers requires the analysis of a large number of clinical samples.

4.5. Cell surface receptors

EGFR protein overexpression has been reported in 70–90% of cases of cervical cancer and associated with poor prognosis either as a single marker or combined with others such as COX-2 [182–185]. An Immunohistochemical assessment of EGFR in HPV-pre-malignant lesions was recently performed by Balan and Coworkers, [186] and they demonstrated that EGFR was overexpressed in 67% of HSIL (56% CIN2 and 43% CIN3) and 32% LSIL, suggesting EGFR was associated with the progression of squamous intraepithelial lesions. However, in previous studies this association was less clear [187,188] and a lack of a consistent link in the studies may result in bias. This means that further studies are necessary to quantify the value of EGFR, as a prognostic factor. One study evaluated the association of HPV-16 E5 and E6 mRNAs expression with EGFR mRNA overexpression, as well as a poor survival rate in cervical cancer. However, just E6 mRNA expression, but not E5 mRNA was linked to overexpression of EGFR [189]. The authors suggest that the lack of association with E5 mRNA could be attributed to the fact that this oncoprotein plays a predominant role in early carcinogenesis [190]. With regard to another surface receptor modulated by HPV E5, the ETAR, only two studies, to date, have referred to its potential as a therapeutic target, which suggests that ETAR-specific blockers could assist in the treatment of cervical cancer [191,192].

4.6. $p21^{Waf1/Sdi1/Cip1}$ e $p27^{KIP1}$

Some studies have shown that p21 and p27 tumor suppressor proteins, which are both inhibited by E5, may be potential prognostic biomarkers for cervical cancer. The expression of p21 has had a favorable prognosis in several types of cancer [193,194]. The patterns of p21 expression in cervical cancer vary considerably, showing both have increased [195–198] and reduced expression [199,200]. Subsequent studies shown cervical tumors with low or absent p27 expression which can be correlated with increased aggressiveness and lymph node metastasis [201,202]. The most recent study, to date, has shown low p27 expression in the prognosis of early cervical carcinoma using univariate and multivariate analyses [203]. However, more studies are needed to confirm the usefulness of these proteins as biomarkers for prognosis in cervical cancer.

4.7. COX-2, VEGF e Cav-1

High levels of COX-2 found in CIN and cervical cancer probably induce tumor progression by a resulting increase of prostaglandins [52,204]. Many studies have shown that COX-2, (regulated by EGFR), is linked to cervical carcinogenesis [205–209]. COX-2 has been cited as causing resistance to both chemotherapy and radiotherapy, an increase of lymph node metastasis and a poor survival rate in cervical cancer [210,211]. Furthermore, Saldivar and colleagues [212] found a concentration 4.9 fold higher with COX-2 in CIN1-2 biopsies than with normal biopsies. However they highlighted the fact that larger sample sizes are required to carry out further evaluations of the role played by COX-2 in the development of premalignant lesions and its value as a biomarker for use in chemoprevention trials. Huang and coworkers [213], recently published the results of a recent meta-analysis in which they provide more precise estimates of the prognostic value of COX-2 in cervical cancer and they concluded that its overexpression might be an unfavorable prognostic factor or a means of predicting chemoradiation resistance for cervical cancer.

Overexpression of VEGF, which is also regulated by activating EGFR, is observed in solid tumors (including colon, lung, and ovarian tumors) [214–216]. In cervical cancer, VEGF was also found to be overexpressed and resulting from poor prognosis [217–221]. Although there have only been a limited number of studies with VEGF and cervical cancer, other types of cancer have been tested by adopting therapeutic approaches with VEGF inhibitors and these have shown promising results such as for the breast [222] and lung [223]. Branca and colleagues [224] showed that VEGF expression increased linearly, starting from low grade neoplasia or CINI and progressing to cervical cancer, which suggests its potential as a predictive marker and as a therapeutic target for early cervical carcinogenesis. Accordingly, current studies have reported an elevated VEGF expression which can be related to poor prognosis in cervical cancer [225] as well as its value as an attractive molecular target that can lead to promising therapeutic strategies to address the problem of this malignancy [226].

The Cav-1 overexpression in E5-expressing cervical cells and its well-known association with other cancers [227] are data that show the need for further studies regarding its potential as a diagnostic marker and therapeutic target of cervical cancer [228]. However, few research studies have evaluated the Cav-1 expression in cervical carcinoma samples. A recent study showed positive rates of Cav-1 in normal cervical mucosa, CIN and cervical cancer of 0%, 33%, and 55%, respectively, suggesting that Cav-1 may be a promoter of cell proliferation in cervical cancer and thus a potential biomarker [228]. Furthermore, Cav-1 may be linked to the ganglioside GM1, which is also increased by E5, and plays a role in the immunosuppressive activity of HPV infected cells [227]. Interestingly, it was observed that detection of GM1 on the surface of E5-expressing cells by means of fluorescence techniques may be a useful strategy for identifying the early stages of hrHPV infection in Pap smears and other clinical specimens [227].

4.8. miRNAs

An increasing number of studies have highlighted significant correlations between miRNA expression patterns with cervical cancer. Several miRNAs have been shown to be dysregulated in cervical tumors such as miR-21, miR-127, miR-145, miR-143, miR-155, miR-199th, miR-146a, miR-29a, miR-15a, miR-16, miR-214, miR-218, and miR-203 among others [2,21,24,111,112,229–231]. Some of these miRNAs are already described in the literature as being influential in neoplastic processes of a variety of cancers, which may help to clarify their role in cervical carcinogenesis. However, studies with cervical cell lines and clinical samples have been performed to identify and evaluate the diagnostic and prognostic significance of altered expression patterns of miRNAs in cervical cancer.

By comparing normal cervical samples and cervical tumor samples, an initial analysis showed a significant reduction of miR-143 and miR-145 as well as increased expression of miR-21 in tumor tissues [21,229]. Subsequent studies found a similar expression pattern of miR-21, which shows the potential of this miRNA as a biomarker of cervical cancer [87,231,232]. Some researchers also found a reduction of miR-143 and miR-145 expression in cervical cancer [23,57,232]. The study of Liu and colleagues demonstrated that miR-143 overexpression blocks cervical tumor formation in mice which suggests its potential use as a therapeutic target [233].

Other studies have found reduced expression of miR-203 in CIN up to invasive cervical cancer [23,230], as well as an increased expression of its target, ΔNp63, which implies there might be a correlation with cancer progression [234,235]. Dysregulation of miR-200a is another significant factor in cervical cancer. Hu and colleagues [230] demonstrated that the link between this miRNA and the ability to predict survival in patients, suggests that miR-200a expression levels could be useful as a prognosis biomarker of cervical cancer.

To date, only three studies have evaluated miRNA expression in premalignant lesions (CIN1-3) in addition to cervical cancer samples [2,113,232]. For instance, Li and colleagues [113] found that the miR-34a levels are reduced in cervical dysplasia and cancer compared with normal tissue; in CIN2 and 3, compared with CIN1; and in hrHPV

infected tissues compared with uninfected tissues. Hence, the authors suggested that modulation of miR-34a by HPV is an early-onset event in the development of cervical cancer in which potential biomarkers may be involved. Similarly, Deftereous and colleagues [232] identified a close link between increased expression of miR-21 and histological diagnosis and suggested that this miRNA is a potential early predictor of tumor progression in cervical carcinogenesis. In another study that included pre-malignant lesions [23], eight miRNAs showed reduced expression during the transition from normal cervix to atypical dysplasia to cancer (miR-26a, miR-143, miR-145, miR-99a, miR-203, miR-513, miR-29a, miR-199a), and five miRNAs displayed increased expression in the transition from normal cervix to atypical dysplasia to cancer (miR-148a, miR-302b, miR-10a, miR-196a and miR-132). However, in most of the published studies discrepancies have been found in the results. The fact that there is a lack of consistent miRNA patterns to distinguish cervical dysplasias and cancer, may be due to variations found in the normal cervical tissue used as control [24,230], or even the different platforms and methods employed by laboratories. In view of this, it will be necessary to perform further validation in larger samples sizes or in multiple cohorts before miRNAs can be introduced into clinical paractice as diagnostic and prognostic biomarkers.

5. Other forms of biomarkers

In addition to the use of biomarkers for the expression levels of genes, proteins and miRNAs, other types of markers have been studied in cervical cancer that are concerned with epigenetic events (methylation of CpG islands in promoter regions) and genetic events (amplifications, deletions, insertions or translocations) [236,237].

The methylation of several important genes for cellular mechanisms is described in cervical cancer [238,239]. These studies have not determined a pattern of methylation in a wide range of samples; however detection of methylation is a useful test that can be used as an adjunct to the Pap smear for cervical cancer screening [240]. Recent papers have been seeking to demonstrate that detecting methylated tumor suppressor genes was a more accurate procedure than HPV DNA testing, since it showed a higher degree of sensitivity and specificity when identifying HSIL in triage of women with ASCUS [241,242]. Other studies showed that methylation analysis may serve as an alternative molecular triage tool for hrHPV positive women, and be able to detect more CIN3 cases than hrHPV testing carried out in combination with conventional cytology [243] and that it increases specificity from 33% to 78% for the detection of CIN3 [244]. With regard to miRNAs, Botezatu and colleagues [245] showed hypermethylation of miR-124a and miR-203 in precursor lesions, and suggested that this may plays a role in cervical oncogenesis.

Aberrations involving chromosome 3 are important genetic events in cervical cancer [246–248]. The gain of chromosome 3q is highly prevalent in cervical cancer-about 70% and has been shown to be associated with the transition from pre-malignant CIN to invasive disease [246,249–251]. Amplification of important genes located in this region, such as hTERC and/or polysomy of chromosome 3, have been detected by recent diagnostic tests using fluorescent *in-situ* hybridization (FISH) technology. These tests have been carried out by some laboratories such as NeoDiagnostix laboratory (Rockville, MD), and the Quest Diagnostics Nichols Institute. This may be an adjunct to cytology screening and HPV testing, since it can identify which LSIL and ASCUS HPV + patients may be at risk of progressing to HSIL and cancer. However, these tests have not yet been cleared or approved by the U.S. Food and Drug Administration (FDA).

The single nucleotide polymorphisms (SNPs) are the genetic events that are most widely studied since high-throughput molecular biological facilities are available [252]. Several works have discussed the presence and association of genetic polymorphisms in both the host genes and HPV genes with a susceptibility to HPV infection and cervical cancer (as investigated by Freitas and coworkers) [253]. However, further functional studies should be conducted to determine the real significance of these polymorphisms [254,255] in the susceptibility and development of cervical cancer.

6. Concluding remarks

Cervical cancer remains a leading cause of morbidity and mortality for women worldwide. There is a need for robust biomarkers to optimize screening methods and treatments, especially in developing countries where a large number of women are already infected by HPV and mortality is closely linked to late diagnosis of neoplasias. The results of current cyto-histological tests show low specificity in predicting the risk of progressive premalignant lesions and as a result affected women have been subjected to under- or over-treatment.

The molecular mechanisms that are promoted by HPV infection, are necessary for the development of cervical cancer and reveal several biomarkers which have a potential use in diagnosis and prognosis. This review has highlighted the HPV-related biomarkers for the improvement of cervical cancer screening that have so far been evaluated. The currently investigations of some of these biomarkers require further tests to validate their routine use. However major immunohistochemical findings involving molecules such as p16 and Ki-67, have shown their potential use in detecting grades of CIN (CIN 1, 2 or 3), risk and progression, as well as making recommendations for the correct clinical management of affected women. In addition, the evaluation of miRNAs and other potential biomarkers, such as the molecular targets of HPV E5, opens up new horizons for cervical cancer detection and therapy.

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References

[1] D.M. Parkin, The global health burden of infection-associated cancers in the year, Int. J. Cancer 118 (2006) 3030–3044.
[2] C.M. Lowndes, Vaccines for cervical cancer, Epidemiol. Infect. 134 (2006) 1–12.
[3] WHO/ICO, Information Centre on HPV and Cervical Cancer (HPV Information Centre), Human Papillomavirus and Related Cancers in World Summary Report, 2010, (Available at http://www.who.int/hpvcentre, accessed 17 March 2013).
[4] I.H. Frazer, Prevention of cervical cancer through papillomavirus vaccination, Nat. Rev. Immunol. 4 (2004) 46–54.
[5] H. zur Hausen, Papillomaviruses and cancer: from basic studies to clinical application, Nat. Rev. Cancer 2 (2002) 342–350.
[6] F.X. Bosh, S. de Sanjose, Chapter 1: human papillomavirus and cervical cancer-burden and assessment of causality, J. Natl. Cancer Inst. Monogr. 31 (2003) 3–13.
[7] N. Munoz, X. Castellsague, A.B. de Gonzalez, L. Gissmanl, Chapter 1: HPV in the etiology of human cancer, Vaccine 24 (2006) 1–10.
[8] H.U. Bernard, R.D. Burk, Z. Chen, K. van Doorslaer, Hz. Hausen, E.M. de Villiers, Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments, Virology 401 (2010) 70–79.
[9] S.F.M. Derchain, A. Longatto Filho, K.J. Syrjanen, Neoplasia intra-epitelial cervical: diagnóstico e tratamento, Rev. Bras. Ginecol. Obstet. 27 (2005) 425–433.
[10] A.P. Pinto, M. Degen, L.L. Villa, E.S. Cibas, Immunomarkers in gynecologic cytology: the search for the ideal biomolecular Papanicolaou test, Acta Cytol. 56 (2012) 109–121.
[11] H.Z. Hausen, Papillomaviruses causing cancer: evasion from host-cell control in early events in carcinogenesis, J. Natl. Cancer Inst. 92 (2000) 690–698.
[12] D. Saslow, D. Solomon, H.W. Lawson, M. Killackey, S.L. Kulasingam, J. Cain, F.A. Garcia, A.T. Moriarty, A.G. Waxman, D.C. Wilbur, N. Wentzensen, L.S. Downs Jr., M. Spitzer, A.B. Moscicki, E.L. Franco, M.H. Stoler, M. Schiffman, P.E. Castle, E.R. Myers, ACS-ASCCP-ASCP Cervical Cancer Guideline Committee, American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer, CA Cancer J. Clin. 62 (2012) 147–172.
[13] G. Ronco, N. Segnan, P. Giorgi-Rossi, M. Zappa, G.P. Casadei, F. Carozzi, P. Dalla Palma, A. Del Mistro, S. Folicaldi, A. Gillio-Tos, G. Nardo, C. Naldoni, P. Schincaglia, M. Zorzi, M. Confortini, J. Cuzick, Human papillomavirus testing and liquid-based cytology: results at recruitment from the new technologies for cervical cancer randomized controlled trial, J. Natl. Cancer Inst. 98 (2006) 765–774.
[14] M. Stanley, Genital human papillomavirus infection-current and prospective therapies, J. Natl. Cancer Inst. Monogr. 31 (2003) 117–124.

[15] C.A. Brown, J. Bogers, S. Sahebali, C.E. Depuydt, F. De Prins, D.P. Malinowski, Role of protein biomarkers in the detection of high-grade disease in cervical cancer screening programs, J. Oncol. 28 (2012) 1–11.
[16] P. Baldwin, R. Laskey, N. Coleman, Translational approaches to improving cervical screening, Nat. Rev. Cancer 3 (2003) 217–226.
[17] M.D. Beccati, C. Buriani, M. Pedriali, S. Rossi, I. Nenci, Quantitative detection of molecular markers ProEx C (minichromosome maintenance protein 2 and topoisomerase IIa) and MIB-1 in liquid-based cervical squamous cell cytology, Cancer 114 (2008) 196–203.
[18] C.O. Sung, S.R. Kim, Y.L. Oh, S.Y. Song, The use of p16 INK4A immunocytochemistry in 'Atypical squamous cells which cannot exclude HSIL' compared with 'Atypical squamous cells of undetermined significance' in liquid-based cervical smears, Diagn. Cytopathol. 38 (2010) 168–171.
[19] A.P. Pinto, C.P. Crum, M.S. Hirsch, Molecular markers of early cervical neoplasia, Diagn. Histopathol. 16 (2010) 445–454.
[20] M. Nasioutziki, A. Daniilidis, K. Dinas, M. Kyrgiou, G. Valasoulis, P.D. Loufopoulos, E. Para-skevaidis, A. Loufopoulos, P. Karakitsos, The evaluation of p16INK4a immune expression/immunostaining and human papillomavirus DNA test in cervical liquid-based cytological samples, Int. J. Gynecol. Cancer 21 (2011) 79–85.
[21] J.W. Lee, C.H. Choi, J.J. Choi, Y.A. Park, S.J. Kim, S.Y. Hwang, W.Y. Kim, J.H. Lee, B.G. Kim, D.S. Bae, Altered MicroRNA expression in cervical carcinomas, Clin. Cancer Res. 14 (2008) 2535–2542.
[22] X. Wang, H.K. Wang, J.P. McCoy, N.S. Banerjee, J.S. Rader, T.R. Broker, C. Meyers, L.T. Chow, Z.M. Zheng, Oncogenic HPV infection interrupts the expression of tumor-suppressive miR-34a through viral oncoprotein E6, RNA 15 (2009) 637–647.
[23] P.M. Pereira, J.P. Marques, A.R. Soares, L. Carreto, M.A. Santos, MicroRNA expression variability in human cervical tissues, PLoS One 5 (2010) e11780.
[24] Q. Rao, H. Zhou, Y. Peng, J. Li, Z. Lin, Aberrant MicroRNA expression in human cervical carcinomas, Med. Oncol. 29 (2012) 1242–1248.
[25] Z.M. Zheng, X. Wang, Regulation of cellular miRNA expression by human papillomaviruses, Biochim. Biophys. Acta 1809 (2011) 668–677.
[26] R.C. Friedman, K.K. Farh, C.B. Burge, D.P. Bartel, Most mammalian mRNAs are conserved targets of microRNAs, Genome Res. 19 (2009) 92–105.
[27] J. Takamizawa, H. Konishi, K. Yanagisawa, S. Tomida, H. Osada, H. Endoh, T. Harano, Y. Yatabe, M. Nagino, Y. Nimura, T. Mitsudomi, T. Takahashi, Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival, Cancer Res. 64 (2004) 3753–3756.
[28] J. Lu, G. Getz, E.A. Miska, E. Alvarez-Saavedra, J. Lamb, D. Peck, A. Sweet-Cordero, B.L. Ebert, R.H. Mak, A.A. Ferrando, J.R. Downing, T. Jacks, H.R. Horvitz, T.R. Golub, MicroRNA expression profiles classify human cancers, Nature 435 (2005) 834–838.
[29] N. Yanaihara, N. Caplen, E. Bowman, M. Seike, K. Kumamoto, M. Yi, R.M. Stephens, A. Okamoto, J. Yokota, T. Tanaka, G.A. Calin, C.G. Liu, C.M. Croce, C.C. Harris, Unique microRNA molecular profiles in lung cancer diagnosis and prognosis, Cancer Cell 9 (2006) 189–198.
[30] A.G. Bader, P.L. Lammers, The Therapeutic Potential of microRNAs, Innov. Pharm. Technol. (2011) 52–55(March Issue).
[31] K. Haller, F. Stubenrauch, H. Pfister, Differentiation-dependent transcription of the epidermodysplasia verruciformis-associated human papillomavirus type 5 in benign lesions, Virology 214 (1995) 245–255.
[32] M.E. McLaughlin-Drubin, K. Munger, Oncogenic activities of human papillomaviruses, Virus Res. 143 (2009) 195–208.
[33] K. Vousden, Interactions of human papillomavirus transforming proteins with the products of tumor suppressor genes, Faseb J. 7 (1993) 872–879.
[34] T. Sandal, Molecular aspects of the mammalian cell cycle and cancer, Oncologist 7 (2002) 73–81.
[35] R.S. Jayshree, A. Sreenivas, M. Tessy, S. Krishna, Cell intrinsic & extrinsic factors in cervical carcinogenesis, Indian J. Med. Res. 130 (2009) 286–295.
[36] C.A. Moody, L.A. Laimins, Human papillomavirus oncoproteins: pathways to transformation, Nat. Rev. Cancer 10 (2010) 550–560.
[37] N. Dyson, P.M. Howley, K. Munger, E. Harlow, The human papilloma virus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product, Science 243 (1989) 934–937.
[38] A.D.S. Eichten, M. Rud, S.O. Grace, V. Piboonniyom, V. Zacny, K. Münger, Molecular pathways executing the "trophic sentinel" response in HPV-16 E7-expressing normal human diploid fibroblasts upon growth factor deprivation, Virology 319 (2004) 81–93.
[39] P. Finzer, A. Aguilar-Lemarroy, F. Rosl, The role of human papillomavirus oncoproteins E6 and E7 in apoptosis, Cancer Lett. 188 (2002) 15–24.
[40] S. Motoyama, C.A. Ladnines-Llave, S.L. Villanueva, T. Maruo, The role of human papilloma virus in the molecular biology of cervical carcinogenesis, Kobe J. Med. Sci. 50 (2004) 9–19.
[41] Y. Ueda, T. Enomoto, T. Miyatake, K. Ozaki, T. Yoshizaki, H. Kanao, Y. Ueno, R. Nakashima, K. Shroyer, Y. Murata, Monoclonal expansion with integration of high-risk type human papillomaviruses is an initial step for cervical carcinogenesis: association of clonal status and human papillomavirus infection with clinical outcome in cervical intraepithelial neoplasia, Lab. Investig. 83 (2003) 1517–1527.
[42] F.X. Bosch, N. Muñoz, K.V. Shah, A. Meheus, Second International Workshop on the epidemiology of cervical cancer and human papillomavirus, Int. J. Cancer 52 (1992) 171–173.
[43] S. Jeon, B.L. Allen-Hoffmann, P.F. Lambert, Integration of human papillomavirus type 16 into the human genome correlates with a selective growth advantage of cells, J. Virol. 69 (1995) 2989–2997.
[44] J. Doorbar, The papillomavirus life cycle, J. Clin. Virol. 32 (2005) 7–15.
[45] S. Duensing, K. Munger, The human papillomavirus type 16 E6 and E7 oncoproteins independently induce numerical and structural chromosome instability, Cancer Res. 62 (2002) 7075–7082.
[46] A. Duensing, Y. Liu, S.A. Perdreau, J. Klevlein-Sohn, E.A. Nigg, S. Duensing, Centriole overduplication through the concurrent formation of multiple daughter centrioles at single maternal templates, Oncogene 26 (2007) 6280–6288.
[47] T.O. Garnett, M. Filippova, P.J. Duerksen-Hughes, Accelerated degradation of FADD and procaspase 8 in cells expressing human papilloma virus 16 E6 impairs TRAIL-mediated apoptosis, Cell Death Differ. 13 (2006) 1915–1926.
[48] T.O. Garnett, P.J. Duerksen-Hughes, Modulation of apoptosis by human papillomavirus (HPV) oncoproteins, Arch. Virol. 151 (2006) 2321–2335.
[49] D. Storey, D. Pim, A. Murray, K. Osborn, L. Banks, L. Crawford, Comparison of the in vitro transforming activities of human papillomavirus types, Embo J. 7 (1988) 1815–1820.
[50] B.A. Werness, A.J. Levine, P.M. Howley, Association of human papillomavirus types 16 and 18 E6 proteins with p53, Science 248 (1990) 76–79.
[51] D. Dimaio, D. Mattoon, Mechanisms of cell transformation by papillomavirus E5 proteins, Oncogene 20 (2001) 7866–7873.
[52] A. Venuti, D. Salani, F. Poggiali, V. Manni, A. Bagnato, The E5 oncoprotein of human papillomavirus type 16 enhances endothelin-1-induced keratinocyte growth, Virology 248 (1998) 1–5.
[53] J.M. Oh, S.H. Kim, Y.I. Lee, M. Seo, S.Y. Kim, Y.S. Song, W.H. Kim, Y.S. Juhnn, Human papillomavirus E5 protein induces expression of the EP4 subtype of prostaglandin E2 receptor in cyclic AMP response element-dependent pathways in cervical cancer cells, Carcinogenesis 30 (2009) 141–149.
[54] E.W. Pedroza-Saavedra, F. Lam, L. Esquivel-Guadarrama, Gutierrez-Xicotencatl, The human papillomavirus type 16 E5 oncoprotein synergizes with EGF-receptor signaling to enhance cell cycle progression and the down-regulation of p27(Kip1), Virology 400 (2010) 44–52.
[55] A.J. Dannenberg, S.M. Lippman, J.R. Mann, K. Subbaramaiah, R.N. DuBois, Cyclooxygenase-2 and epidermal growth factor receptor: pharmacologic targets for chemoprevention, J. Clin. Oncol. 23 (2005) 254–266.
[56] G.A. Calin, C. Sevignani, C.D. Dumitru, T. Hyslop, E. Noch, S. Yendamuri, M. Shimizu, S. Rattan, F. Bullrich, M. Negrini, C.M. Croce CM, Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 2999–3004.
[57] C.B. Lajer, E. Garnæs, L. Friis-Hansen, B. Norrild, M.H. Therkildsen, M. Glud, M. Rossing, H. Lajer, D. Svane, L. Skotte, L. Specht, C. Buchwald, F.C. Nielsen, The role of miRNAs in human papilloma virus (HPV)-associated cancers: bridging between HPV-related head and neck cancer and cervical cancer, Br. J. Cancer 106 (2012) 1526–1534.
[58] G. Reshmi, M. Radhakrishna Pillai, in: D. Vanden Broeck (Ed.), Interplay Between HPV Oncoproteins and MicroRNAs in Cervical Cancer book, Published: January 25, 2012 under CC BY 3.0 license Chapter 14 Interplay Between HPV Oncoproteins and MicroRNAs in Cervical Cancer, ISBN: 978-953-307-855-7, 2012, http://dx.doi.org/10.5772/29866.
[59] N. Greco, K. Kivi, S.K. Qian, P. Leivonen, E. Auvinen, Human papillomavirus 16 E5 modulates the expression of host microRNAs, PLoS One 6 (2011) e21646.
[60] T. Rajkumar, K. Sabitha, N. Vijayalakshmi, S. Shirley, M.V. Bose, G. Gopal, G. Selvaluxmy, Identification and validation of genes involved in cervical tumourigenesis, BMC Cancer 11 (2011) 1–14.
[61] S.J. wang, K.R. Shroyer, Biomarkers of cervical dysplasia and carcinoma, J. Oncol. (2012) 1–9.
[62] K. Munger, W.C. Phelps, V. Bubb, P.M. Howley, R. Schlegel, The E6 and E7 genes of the human papillomavirus type 16 together are necessary and sufficient for transformation of primary human keratinocytes, J. Virol. 63 (1989) 4417–4421.
[63] T. Sano, T. Oyama, K. Kashiwabara, T. Fukuda, T. Nakajima, Expression status of p16 protein is associated with human papillomavirus oncogenic potential in cervical and genital lesions, Am. J. Pathol. 153 (1998) 1741–1748.
[64] V. Bouvard, G. Matlashewski, Z.M. Gu, A. Storey, L. Banks, The human papillomavirus type 16 E5 gene cooperates with the E7 gene to stimulate proliferation of primary cells and increases viral gene expression, Virology 203 (1994) 73–80.
[65] S.M.G. Williams, G.L. Disbrow, R. Schlegel, D. Lee, D.W. Threadgill, P.F. Lambert, Requirement of epidermal growth factor receptor for hyperplasia induced by E5, a high-risk human papillomavirus oncogenes, Cancer Res. 65 (2005) 6534–6542.
[66] F.A. Suprynowicz, M.S. Campo, R. Schlegel, Biological activitites of papillomavirus E5 proteins, in: M.S. Campo (Ed.), Papillomavirus Research: From Natural History to Vaccines and Beyond, Caister Academic Press, Wymondham, United Kingdom, 2006, pp. 97–113.
[67] Z. Sahab, S.R. Sudarshan, X. Liu, Y. Zhang, A. Kirilyuk, C.M. Kamonjoh, V. Simic, Y. Dai, S.W. Byers, J. Doorbar, F.A. Suprynowicz, R. Schlegel, Quantitative measurement of human papillomavirus type 16 E5 oncoprotein levels in epithelial cell lines by mass spectrometry, J. Virol. 86 (2012) 9465–9947.
[68] K.B. Choo, C.C. Pan, M.S. Liu, S.H. Han, H.T. Ng, C.P. Chen, Y.N. Lee, C.F. Chao, C.L. Meng, M.Y. Yeh, Presence of episomal and integrated human papilomavirus DNA sequences in cervical carcinoma, J. Med. Virol. 21 (1987) 101–107.
[69] A.P. Cullen, R. Reid, M. Campion, A.T. Lorincz, Analysis of the physical state of different human papillomavirus DNAs in intraepithelial and invasive cervical neoplasm, J. Virol. 65 (1991) 606–612.
[70] J.L. Chang, Y.P. Tsao, D.W. Liu, S.J. Huang, W.H. Lee, S.L. Chen, The expression of HPV-16 E5 protein in squamous neoplastic changes in the uterine cervix, J. Biomed. Sci. 8 (2001) 206–213.
[71] N. Hafner, C. Driesch, M. Gajda, L. Jansen, R. Kirchmayr, I.B. Runnebaum, M. Dürst, Integration of the HPV16 genome does not invariably result in high levels of viral oncogene transcripts, Oncogene 27 (2008) 1610–1617.
[72] J. Koh, G.H. Enders, B.D. Dynlacht, E. Harlow, Tumour-derived p16 alleles encoding proteins defective in cell-cycle inhibition, Nature 375 (1995) 506–510.
[73] S.N. Khleif, J. Degregori, C.L. Yee, G.A. Otterson, F.J. Kaye, J.R. Nevins, P.M. Howley, Inhibition of cyclin D-CDK4/CDK6 activity is associated with an E2F-mediated

induction of cyclin kinase inhibitor activity, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 4350–4354.
[74] R. Klaes, T. Friedrich, D. Spitkovsky, R. Ridder, W. Rudy, U. Petry, G.D. Hellweg, D. Schmidt, M.V.K. Doeberitz, Overexpression of p16INK4a as a specific marker for dysplastic and neoplastic epithelial cells of the cervix uteri, Int. J. Cancer 92 (2001) 276–284.
[75] S. Nieh, S.F. Chen, T.Y. Chu, H.C. Lai, E. Fu, Expression of p16INK4A in Papanicolaou smears containing atypical squamous cells of undetermined significance from the uterine cervix, Gynecol. Oncol. 91 (2003) 201–208.
[76] M. Guo, L. Hu, M. Baliga, Z. He, M.D. Hughson, The predictive value of p16INK4a and hybrid capture 2 human papillomavirus testing for high-grade cervical intraepithelial neoplasia, Am. J. Clin. Pathol. 122 (2004) 894–901.
[77] S. Nieh, S.F. Chen, T.Y. Chu, H.C. Lai, E. Fu, C.H. Gau, Is p16INK4A expression more useful than human papillomavirus test to determine the outcome of atypical squamous cells of undetermined significance-categorized Pap smear? A comparative analysis using abnormal cervical smears with follow-up biopsies, Gynecol. Oncol. 97 (2005) 35–40.
[78] T. Scholzen, J. Gerdes, The Ki-67 protein: from the known and the unknown, J. Cell. Physiol. 182 (2000) 311–322.
[79] A.J. Kruse, J.P.A. Baak, E.A. Janssen, K.H. Kjellevold, B. Fiane, K. Lovslett, J. Bergh, S. Robboy, Ki67 predicts progression in early CIN: validation of a multivariate progression-risk model, Cell. Oncol. 26 (2004) 13–20.
[80] J.P. Baak, A.J. Kruse, S.J. Robboy, E.A. Janssen, B.V. Diermen, I. Skaland, Dynamic behavioural interpretation of cervical intraepithelial neoplasia with molecular biomarkers, J. Clin. Pathol. 59 (2006) 1017–1028.
[81] A.J. Kruse, J.P. Baak, P.C. de Bruin, M. Jiwa, W.P. Snijders, P.J. Boodt, G. Fons, P.W. Houben, H. S. Ki-67 immunoquantitation in cervical intraepithelial neoplasia (CIN): a sensitive marker for grading, J. Pathol. 193 (2001) 48–54.
[82] A.D. Santin, F. Zhan, E. Bignotti, R.E. Siegelc, S. Canéa, S. Bellonea, M. Palmieria, S. Anfossia, M. Thomas, A. Burnetta, H.H. Kaye, J.J. Romana, T.J. O'Briena, E. Tianb, M.J. Cannonf, J. Shaughnessy Jr., S. Pecorellig, Gene expression profiles of primary HPV16- and HPV18-infected early stage cervical cancers and normal cervical epithelium: identification of novel candidate molecular markers for cervical cancer diagnosis and therapy, Virology 331 (2005) 269–291.
[83] D.P. Malinowski, Molecular diagnostic assays for cervical neoplasia: emerging markers for the detection of high-grade cervical disease, Biotechniques 38 (2005) S17–S23.
[84] G. Carpenter, Receptors for epidermal growth factor and other polypeptide mitogens, Annu. Rev. Biochem. 56 (1987) 881–914.
[85] G. Carpenter, M.I. Wahl, The epidermal growth factor family, in: M.B. Sporn, A.B. Roberts (Eds.), Peptide Growth Factors and their Receptors, Springer-Verlag, Berlin, 1990, pp. 69–171.
[86] S.W. Straight, B. Herman, D.J. McCance, The E5 oncoprotein of human papillomavirus type 16 inhibits the acidification of endosomes in human keratinocytes, J. Virol. 69 (1995) 3185–3192.
[87] K. Crusius, E. Auvinen, B. Steuer, H. Gaissert, A. Alonso, The human papillomavirus type 16 E5-protein modulates ligand-dependent activation of the EGF receptor family in the human epithelial cell line HaCaT, Exp. Cell Res. 241 (1998) 76–83.
[88] S.D. Chell, I.R. Witherden, R.R. Dobson, M. Moorghen, A.A. Herman, D. Qualtrough, A.C. Williams, C. Paraskeva, Increased EP4 receptor expression in colorectal cancer progression promotes cell growth and anchorage independence, Cancer Res. 66 (2006) 3106–3113.
[89] M.K. Kim, H.S. Kim, S.H. Kim, J.M. Oh, J.Y. Han, J.M. Lim, Y.S. Juhnn, Y.S. Song, Human papillomavirus type 16 E5 oncoprotein as a new target for cervical cancer treatment, Biochem. Pharmacol. 80 (2010) 1930–1935.
[90] Y.P. Tsao, L.Y. Li, T.C. Tsai, S.L. Chen, Human papillomavirus type 11 and 16 E5 represses p21(Wafl/Sdil/Cipl) gene expression in fibroblasts and keratino-cytes, J. Virol. 70 (1996) 7535–7539.
[91] W.L. Smith, D.L. DeWitt, R.M. Garavito, Cyclooxygenases: structural, cellular, and molecular biology, Annu. Rev. Biochem. 69 (2000) 145–182.
[92] C.S. Williams, M. Tsujii, J. Reese, S.K. Dey, R.N. DuBois, Host cyclooxygenase-2 modulated carcinoma growth, J. Clin. Investig. 105 (2000) 1589–1594.
[93] D.J. Elder, D.E. Halton, A. Hague, C. Paraskeva, Induction of apoptotic cell death in human colorectal carcinoma cell lines by a cyclooxygenase-2 (COX-2)-selective nonsteroidal anti-inflammatory drug: independence from COX-2 protein expression, Clin. Cancer Res. 3 (1997) 1679–1683.
[94] J.L. Masferrer, K.M. Leahy, A.T. Koki, B.S. Zweifel, S.L. Settle, B.M. Woerner, D.A. Edwards, A.G. Flickinger, R.J. Moore, K. Seibert, Antiangiogenic and antitumor activities of cyclooxygenase-2 inhibitors, Cancer Res. 60 (2000) 1306–1311.
[95] H.J. Kang, G. Gong, S.J. Jang, P.J. Jung, C.K. Park, Expression of cyclooxygenase-2 in human breast carcinoma: relevance to tumor angiogenesis and expression of estrogen receptor, Cancer Res. Treat. 33 (2001) 286–295.
[96] A.M. Byrne, D.J. Bouchier-Hayes, J.H. Harmey, Angiogenic and cell survival functions of vascular endothelial growth factor (VEGF), J. Cell. Mol. Med. 9 (2005) 777–794.
[97] S.H. Kim, Y.S. Juhnn, S. Kang, S.W. Park, M.W. Sung, Y.J. Bang, Human papillomavirus 16 E5 up-regulates the expression of vascular endothelial growth factor through the activation of epidermal growth factor receptor MEK/ERK1,2 and PI3K/Akt, Cell. Mol. Life Sci. 63 (2006) 930–938.
[98] S.H. Kim, J.M. Oh, J.H. No, Y.J. Bang, Y.S. Juhnn, Y.S. Song, Involvement of NF-kappaB and AP-1 in COX-2 upregulation by human papillomavirus 16 E5 oncoprotein, Carcinogenesis 30 (2009) 753–757.
[99] D. Hanahan, J. Folkman, Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis, Cell 86 (1996) 353–364.
[100] R.G. Parton, Caveolae and caveolins, Curr. Opin. Cell Biol. 8 (1996) 542–548.
[101] E.J. Smart, A.G. Graf1, M.A. McNiven, W.C. Sessa, J.A. Engelman, P.E. Scherer, T. Okamoto, M.P. Lisanti, Caveolins, liquid-ordered domains, and signal transduction, Mol. Cell. Biol. 19 (1999) 7289–7304.
[102] A. Abulrob, S. Giuseppin, M.F. Andrade, A. McDermid, M. Moreno, D. Stanimirovic, Interactions of EGFR and caveolin-1 in human glioblastoma cells: evidence that tyrosine phosphorylation regulates EGFR association with caveolae, Oncogene 23 (2004) 6967–6979.
[103] M.O. Parat, G.J. Riggins, Caveolin-1, caveolae, and glioblastoma, Neuro Oncol. 14 (2012) 679–688.
[104] D.P. Bartel, MicroRNAs: target recognition and regulatory functions, Cell 136 (2009) 215–233.
[105] S. Vasudevan, Functional validation of microRNA-target RNA interactions, Methods 58 (2012) 126–134.
[106] B.D. Harfe, MicroRNAs in vertebrate development, Curr. Opin. Genet. Dev. 15 (2005) 410–415.
[107] A.M. Gurtan, P.A. Sharp, The role of miRNAs regulating gene expression, J. Mol. Biol. 425 (2013) 3582–3600.
[108] G. Reshmi, M.R. Pillai, Interplay Between HPV Oncoproteins and MicroRNAs in Cervical Cancer, in: D.V. Broeck (Ed.), Human Papillomavirus and Related Diseases—From Bench to Bedside—Research aspects, InTech., 2012, pp. 349–356.
[109] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell 116 (2004) 281–297.
[110] M. Negrini, M.S. Nicoloso, G.A. Calin, MicroRNAs and cancer–new paradigms in molecular oncology, Curr. Opin. Cell Biol. 21 (2009) 470–479.
[111] I. Martinez, A.S. Gardiner, K.F. Board, F.A. Monzon, R.P. Edwards, S.A. Khan, Human papillomavirus type 16 reduces the expression of microRNA-218 in cervical carcinoma cells, Oncogene 27 (2008) 2575–2582.
[112] X. Wang, S. Tang, S.Y. Le, R. Lu, J.S. Rader, C. Meyers, Z.M. Zheng, Aberrant expression of oncogenic and tumor-suppressive microRNAs in cervical cancer is required for cancer cell growth, PLoS One 3 (2008) e2557.
[113] B. Li, Y. Hu, F. Ye, Y. Li, W. Lv, X. Xie, Reduced miR-34a expression in normal cervical tissues and cervical lesions with high-risk human papillomavirus infection, Int. J. Gynecol. Cancer 20 (2010) 597–604.
[114] L. He, X. He, L.P. Lim, E. de Stanchina, Z. Xuan, Y. Liang, W. Xue, L. Zender, J. Magnus, D. Ridzon, A microRNA component of the p53 tumour suppressor network, Nature 447 (2007) 1130–1134.
[115] N. Raver-Shapira, E. Marciano, E. Meiri, Y. Spector, N. Rosenfeld, N. Moskovits, Z. Bentwich, M. Oren, Transcriptional activation of miR-34a contributes to p53-mediated apoptosis, Mol. Cell 26 (2007) 731–743.
[116] G.T. Bommer, I. Gerin, Y. Feng, A.J. Kaczorowski, R. Kuick, R.E. Love, Y. Zhai, T.J. Giordano, Z.S. Qin, B.B. Moore, p53-mediated activation of miRNA34 candidate tumor-suppressor genes, Curr. Biol. 17 (2007) 1298–1307.
[117] H. Tazawa, N. Tsuchiya, M. Izumiya, H. Nakagama, Tumor-suppressive miR-34a induces senescence-like growth arrest through modulation of the E2F pathway in human colon cancer cells, Proc. Natl. Acad. Sci. 104 (2007) 15472–15477.
[118] T. Yao, Lin, MiR-21 is involved in cervical squamous cell tumorigenesis and regulates CCL20, Biochim. Biophys. Acta 1822 (2012) 248–260.
[119] M. Melar-New, A. Laimins, Human papillomaviruses modulate expression of microRNA 203 upon epithelial differentiation to control levels of p63 proteins, J. Virol. 84 (2010) 5212–5221.
[120] M.H. Stoler, M. Schiffman, Interobserver reproducibility of cervical cytologic and histologic interpretations: realistic estimates from the ASCUS-LSIL Triage Study, JAMA 285 (2001) 1500–1505.
[121] J. Cuzick, C. Clavel, K.U. Petry, C.J. Meijer, H. Hoyer, S. Ratnam, A. Szarewski, P. Birembaut, S. Kulasingam, P. Sasieni, T. Iftner, Overview of the European and North American studies on HPV testing in primary cervical cancer screening, Int. J. Cancer 119 (2006) 1095–1101.
[122] M. Schiffman, N. Wentzensen, From human papillomavirus to cervical cancer, Obstet. Gynecol. 116 (2010) 177–185.
[123] N. Wentzensen, L. Schwartz, R.E. Zuna, K. Smith, C. Mathews, M.A. Gold, R.A. Allen, R. Zang, S.T. Dunn, J.L. Walker, M. Schiffman, Performance of p16/Ki-67 Immunostaining to Detect Cervical Cancer Precursors in a Colposcopy Referral Population, Clin. Cancer Res. 18 (2012) 4154–4162.
[124] P.E. Castle, D. Solomon, M. Schiffman, C.M. Wheeler, Human papilloma-virus type 16 infections and 2-year absolute risk of cervical precancer in women with equivocal or mild cytologic abnormalities, J. Natl. Cancer Inst. 97 (2005) 1066–1071.
[125] J.L. Walker, S.S. Wang, M.H. Schiffman, D. Solomon, Predicting absolute risk of CIN3 during post-colposcopic follow-up: results from the ASCUS-LSIL Triage Study (ALTS), Am. J. Obstet. Gynecol. 195 (2006) 341–348.
[126] G.S. Ogilvie, M. Krajden, D.J. van Niekerk, R.E. Martin, T.G. Ehlen, K. Ceballos, L.W. Smith, L. Kan, D.A. Cook, S. Peacock, G.C. Stuart, E.L. Franco, A.J. Coldman, Primary cervical cancer screening with HPV testing compared with liquid-based cytology: results of round 1 of a randomised controlled trial—the HPV FOCAL Study, Br. J. Cancer 107 (2012) 1917–1924.
[127] M. Clifford, J.S. Smith, M. Plummer, N. Muñoz, S. Franceschi, Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis, Br. J. Cancer 88 (2003) 63–73.
[128] L. Termini, L. Lina Villa, Biomarcadores na triagem do câncer do colo uterino/Biomarkers in cervical cancer screening DST, J. bras Doenças Sex Transm. 20 (2008) 125–131.
[129] I.H. Frazer, Prevention of cervical cancer through papillomavirus vaccination, Nat. Rev. Immunol. 4 (2004) 46–54.
[130] H. Freeman, B. Wingrove, Excess Cervical Cancer Mortality: A Marker for Low Access to Health Care in Poor Communities, National Cancer Institute, Rockville, MD, 2005.
[131] J.S. Smith, L. Lindsay, B. Hoots, J. Keys, S. Franceschi, R. Winer, R,.G.M. Clifford, Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: A meta-analysis update, Int. J. Cancer 121 (2007) 621–632.

[132] I. Tsoumpou, M. Arbyn, M. Kyrgiou, N. Went-zensen, G. Koliopoulos, P.M. Hirsch, V.M. Mitsi, E. Paraskevaidis, p16 INK4a immunostaining in cytological and histological specimens from the uterine cervix: a systematic review and meta-analysis, Cancer Treat. Rev. 35 (2009) 210–220.

[133] M.Z. Huang, S. Huang, D.Q. Li, X.M. Nie, H.B. Li, X.M. Jiang, The study of the combination detection of HPV-DNA and p16INK4a in cervical Lesions, Med. Oncol. 28 (2011) S547–S552.

[134] P.L. Cheah, L.M. Looi, K.H. Teoh, K.S. Mun, A.R. Nazarina, p16(INK4a) is a useful marker of human papillomavirus integration allowing risk stratification for cervical malignancies, Asian Pac. J. Cancer Prev. 13 (2012) 469–472.

[135] C. Rosamilia, G. Feichter, A. Tzankov, E.C. Obermann, Diagnosis and grading of cervical intraepithelial neoplasias, Pathologe 33 (2012) 118–123.

[136] S. Nishio, T. Fujii, H. Nishio, K. Kameyama, M. Saito, T. Iwata, K. Kubushiro3, D. Aoki, $p16^{INK4a}$ immunohistochemistry is a promising biomarker to predict the outcome of low grade cervical intraepithelial neoplasia: comparison study with HPV genotyping. J, Gynecol. Oncol. 24 (2013) 215–221.

[137] E.A. Portari, F.B. Russomano, M.J. de Camargo, C.R. Machado Gayer, H.C. da Rocha Guillobel, C.B. Santos-Rebouças, J.M. Brito Macedo, Immunohistochemical Expression of Cyclin D1, p16Ink4a, p21WAF1, and Ki-67 Correlates With the Severity of Cervical Neoplasia, Int. J. Gynecol. Pathol. 32 (2013) 501–508.

[138] J. Roelens, M. Reuschenbach, M.V.K. Doeberitz, N. Wentzensen, C. Bergeron, M. Arbyn, p16INK4a immunocytochemistry versus human papillomavirus testing for triage of women with minor cytologic abnormalities: a systematic review and meta-analysis, Cancer Cytopathol. 120 (2012) 294–307.

[139] D. Gustinucci, B. Passamonti, E. Cesarini, D. Butera, E.A. Palmieri, S. Bulletti, A. Carlani, M. Staiano, M.R. D'Amico, V. D'Angelo, E. Di Dato, N. Martinelli, M. Malaspina, N. Spita, B. Tintori, F. Fulciniti, Role of p16(INK4a) cytology testing as an adjunct to enhance the diagnostic specificity and accuracy in human papillomavirus-positive women within an organized cervical cancer screening program, Acta Cytol. 56 (2012) 506–514.

[140] R. Samir, A. Asplund, T. Tot, G. Pekar, D. Hellberg, High-risk HPV infection and CIN grade correlates to the expression of c-myc, CD4+, FHIT, E-cadherin, Ki-67, and p16INK4a, J. Low Genit. Tract Dis. 15 (2011) 280–286.

[141] J.V. Hidalgo, A.E. Rocher, J.L. López, M. Gamboni, S. Vighi, O.E. Canessa, S. Peressini, F. Guerra, M.B. di Carlo, L.A. Palaoro, S.A. Tatti, AgNOR, p16 and human papillomavirus in low-grade squamous intra-epithelial lesions of the uterine cervix. Preliminary report, Biotech. Histochem. 87 (2012) 257–264.

[142] L.J. van Bogaert, P16INK4a imunocytochemistry/immunohistochemistry: need for scoring uniformization to be clinically useful in gynecological pathology, Ann. Diagn. Pathol. 16 (2012) 422–426.

[143] K.D. Quint, M.N. Koning, W.G. Quint, E.C. Pirog, Progression of cervical low grade squamous intraepithelial lesions: in search of prognostic biomarkers, Eur. J. Obstet. Gynecol. Reprod. Biol. S0301–2115 (2013) 00319-9.

[144] Y.T. Kim, M. Zhao, Aberrant cell cycle regulation in cervical carcinoma, Yonsei Med. J. 46 (2005) 597–613.

[145] A.N. Kalof, K. Cooper, p16INK4a immunoexpression: Surrogate markerof high-risk HPV and high-grade cervical intraepithelial neoplasia, Adv. Anat. Pathol. 13 (2006) 190–194.

[146] G.A. Boulet, C.A. Horvath, C.E. Depuydt, J.J. Bogers, Biomarkers in cervical screening: quantitative reverse transcriptase PCR analysis of P16INK4a expression, Eur. J. Cancer Prev. 19 (2010) 35–41.

[147] H. von Keyserling, W. Kühn, A. Schneider, T. Bergmann, A.M. Kaufmann, $p16INK^4a$ and p14ARF mRNA expression in Pap smears is age-related, Mod. Pathol. 25 (2012) 465–470.

[148] M. Omori, A. Hashi, K. Nakazawa, T. Yuminamochi, T. Yamane, S. Hirara, R. Katoh, K. Hoshi, Estimation of prognoses for Cervical Intraepithelial Neoplasia 2 by p16INK4a immunoexpression and High-Risk HPV In Situ Hybridization signal types, Am. J. Clin. Pathol. 128 (2007) 208–217.

[149] G. Negri, G. Bellisano, E. Carico, G. Faa, A. Kasal, S. Antoniazzi, E. Egarter-Vigl, A. Piccin, P. Dalla Palma, F. Vittadello, Usefulness of p16INK4a, ProEX C, and Ki-67 for the diagnosis of glandular dysplasia and adenocarcinoma of the cervix uteri, Int. J. Gynecol. Pathol. 30 (2011) 407–413.

[150] S. Ozaki, Y. Zen, M. Inoue, Biomarker expression in cervical intraepithelial neoplasia: potential progression predictive factors for low-grade lesions, Hum. Pathol. 42 (2011) 1007–1012.

[151] N. Izadi-Mood, K. Asadi, H. Shojaei, S. Sarmadi, S.A. Ahmadi, S. Sani, L.H. Chelav Potential, diagnostic value of P16 expression in premalignant and malignant cervical lesions, J. Res. Med Sci. 17 (2012) 428–433.

[152] C. Schluter, M. Duchrow, C. Wohlenberg, M.H. Becker, G. Key, H.D. Flad, J. Gerdes, The cell proliferation-associated antigen of antibody Ki-67: a very large, ubiquitous nuclear protein with numerous repeated elements, representing a new kind of cell cycle-maintaining proteins, J. Cell Biol. 123 (1993) 513–522.

[153] A.J. Kruse, J.P. Baak, P.C. de Bruin, M. Jiwa, W.P. Snijders, P.J. Boodt, G. Fons, P.W. Houben, The HS Ki-67 immunoquantitation in cervical intraepithelial neoplasia (CIN): a sensitive marker for grading, J. Pathol. 193 (2001) 48–54.

[154] A.J. Kruse, J.P.A. Baak, E.A. Janssen, M.G. Bol, K.H. Kjellevold, B. Fianne, K. Lovslett, J. Bergh, Low- and high-risk CIN 1 and 2 lesions: prospective predictive value of grade, HPV, and Ki-67 immunouantitative variables, J. Pathol. 199 (2003) 462–470.

[155] M. Panjkovic, T. Ivkovic-Kapicl, Ki-67 expression in squamous intraepithelial lesions of the uterine cervix Arch, Oncol. 14 (2006) 23–25.

[156] P. Conesa-Zamora, A. Doménech-Peris, F.J. Orantes-Casado, S. Ortiz-Reina, L. Sahuquillo-Frías, J. Acosta-Ortega, J. García-Solano, M. Pérez-Guillermo, Effect of human papillomavirus on cell cycle-related proteins p16, Ki-67, cyclin D1, p53, and ProEx C in precursor lesions of cervical carcinoma: a tissue microarray study, Am. J. Clin. Pathol. 132 (2009) 378–390.

[157] M.T. Galgano, P.E. Castle, K.A. Atkins, W.K. Brix, S.R. Nassau, M.H. Stoler, Using Biomarkers as Objective Standards in the Diagnosis of Cervical Biopsies, Am. J. Surg. Pathol. 34 (2010) 1077–1087.

[158] M. Mimica, S. Tomić, G. Kardum, I.D. Hofman, V. Kaliterna, L. Pejković, Ki-67 quantitative evaluation as a marker of cervical intraepithelial neoplasia and human papillomavirus infection, Int. J. Gynecol. Cancer 20 (2010) 116–119.

[159] A.J. Kruse, J.P. Baak, P.C. de Bruin, F.R. van de Goot, N. Kurten, Relationship between the presence of oncogenic HPV DNA assessed by polymerase chain reaction and Ki-67 immunoquantitative features in cervical intraepithelial neoplasia, J. Pathol. 195 (2001) 557–562.

[160] E.C. Pirog, R.N. Baergen, R.A. Soslow, D. Tam, A.E. DeMattia, Y.T.M.D. Chen, C.M.D. Isacson, Diagnostic accuracy of cervical low-grade squamous intraepithelial lesions is improved with Ki-67 immunostaining, Am. J. Surg. Pathol. 26 (2002) 70–75.

[161] S. Sahebali, C.E. Depuydt, K. Segers, A.J. Vereecken, E. Van Marck, J.J. Bogers, Ki-67 immunocytochemistry in liquid based cervical cytology: useful as an adjunctive tool? J. Clin. Pathol. 56 (2003) 681–686.

[162] A.J. Kruse, S. Buhr-Wildhagen, E.A. Janssen, J.P. Baak, The relationship between syntactic structure analysis features, histological grade and high-risk HPV DNA in cervical intraepithelial neoplasia, Cell. Oncol. 26 (2004) 135–141.

[163] A.J. Kruse, E. Gudlaugsson, T. Helliesen, E.A. Janssen, B. van Diermen, S. Sandvik, J.P. Baak, Evaluation of prospective, routine application of Ki-67 immunoquantitation in early CIN for assessment of short-term progression risk, Anal. Quant. Cytol. Histol. 26 (2004) 134–140.

[164] M.G. Donà, A. Vocaturo, M. Giuliani, L. Ronchetti, F. Rollo, E. Pescarmona, M. Carosi, G. Vocaturo, M. Benevolo, p16/Ki-67 dual staining in cervico-vaginal cytology: correlation with histology, Human Papillomavirus detection and genotyping in women undergoing colposcopy, Gynecol. Oncol. 126 (2012) 198–202.

[165] D. Schmidt, C. Bergeron, K.J. Denton, R. Ridder, p16/ki-67 dual-stain cytology in the triage of ASCUS and LSIL Papanicolaou cytology: results from the European equivocal or mildly abnormal Papanicolaou cytology study, Cancer Cytopathol. 119 (2011) 158–166.

[166] K.U. Petry, D. Schmidt, S. Scherbring, A. Luyten, A. Reinecke-Lüthge, C. Bergeron, F. Kommoss, T. Löning, J. Ordi, S. Regauer, R. Ridder, Triaging Pap cytology negative, HPV positive cervical cancer screening results with p16/Ki-67 Dual-stained cytology, Gynecol. Oncol. 121 (2011) 505–509.

[167] M. Singh, D. Mockler, A. Akalin, S. Burke, A. Shroyer, K.R. Shroyer, Immunocytochemical colocalization of P16(INK4a) and Ki-67 predicts CIN2/3 and AIS/adenocarcinoma, Cancer Cytopathol. 120 (2012) 26–34.

[168] W. Rokita, D. Skawiński, A. Zmelonek-Znamirowska, W. Kedzia, A. Karowicz-Bilińska, R. Spaczyński, M. Spaczyński, Results of pap smears and immunocytochemical detection of the p16 and Ki67 proteins in women with cervical intraepithelial neoplasia and cervical cancer, Ginekol. Pol. 83 (2012) 822–826.

[169] L. Yu, L. Wang, J. Zhong, S. Chen, Diagnostic value of p16INK4A, Ki-67, and human papillomavirus L1 capsid protein immunochemical staining on cell blocks from residual liquid-based gynecologic cytology specimens, Cancer Cytopathol. 118 (2010) 47–55.

[170] P. Samarawardana, M. Singh, K.R. Shroyer, Dual stain immunohistochemical localization of p16INK4A and ki-67: a synergistic approach to identify clinically significant cervical mucosal lesions, Appl. Immunohistochem. Mol. Morphol. 19 (2011) 514–518.

[171] A. Gertych, A.O. Joseph, A.E. Walts, S. Bose, Automated detection of dual p16/Ki67 nuclear immunoreactivity in liquid-based Pap tests for improved cervical cancer risk stratification, Ann. Biomed. Eng. 40 (2012) 1192–1204.

[172] R.E. Badr, A.E. Walts, F. Chung, S. Bose, BDProExC: a sensitive and specific marker of HPV-associated squamous lesions of the cervix, Am. J. Surg. Pathol. 32 (2008) 899–906.

[173] C.E. Depuydt, A.P. Makar, M.J. Ruymbeke, I.H. Benoy, A.J. Vereecken, J.J. Bogers, BD-ProExC as adjunct molecular marker for improved detection of CIN2+ after HPV primary screening, Cancer Epidemiol. Biomarkers Prev. 20 (2011) 628–637.

[174] K.R. Shroyer, P. Homer, D. Heinz, M. Singh, Validation of a novel immunocytochemical assay for topoisomerase II-α and minichromosome maintenance protein 2 expression incervical cytology, Cancer 108 (2006) 324–330.

[175] D. Kelly, E. Kincaid, Z. Fansler, D.L. Rosenthal, D.P. Clark, Detection of cervical high-grade squamous intraepithelial lesions from cytologic samples using a novel immunocytochemical assay (ProEx C), Cancer 108 (2006) 494–500.

[176] R.H. Tambouret, J. Misdraji, D.C. Wilbur, Longitudinal clinical evaluation of a novel antibody cocktail for detection of high-grade squamous intraepithelial lesions on cervical cytology specimens, Arch. Pathol. Lab. Med. 132 (2008) 918–925.

[177] R. Alaghehbandan, D. Fontaine, J. Bentley, N. Escott, P. Ghatage, A. Lear, F. Coutlee, S. Ratnam, Performance of proex c and pretect hpv-proofer E6/E7 mRNA tests in comparison with the hybrid capture 2 hpv dna test for triaging ASCUS and LSIL cytology, Diagn. Cytopathol. 41 (2013) 767–775.

[178] M.T. Siddiqui, K. Hornaman, C. Cohen, A. Nassar, ProEx C immunocytochemistry and high-risk human papillomavirus DNA testing in papanicolaou tests with atypical squamous cell (ASC-US) cytology: correlation study with histologic biopsy, Arch. Pathol. Lab. Med. 132 (2008) 1648–1652.

[179] D. Aximu, A. Azad, R. Ni, T. Colgan, S. Nanji, A pilot evaluation of a novel immunohistochemical assay for topoisomerase II-α and minichromosome maintenance protein 2 expression (ProEx C) in cervical adenocarcinoma in situ, adenocarcinoma, and benign glandular mimics, Int. J. Gynecol. Pathol. 28 (2009) 114–119.

[180] T. Goto, M. Takano, H. Sasa, H. Tsuda, K. Yamau-chi, Y. Kikuchi, Clinical significance of immunocytochemistry for PIK3CA as a carcinogenesis-related marker on liquid-based cytology in cervical intraepithelial neoplasia, Oncol. Rep. 15 (2006) 387–391.

[181] M.I. Micalessi, G. Boulet, I. Pintelon, P. Verstraelen, F. Nauwelaers, M. Ryser, J. Bogers, High-Content Imaging in Cervical Cancer Screening, J. Biomol. Screen. 18 (1) (2013) 135–142.

[182] R.I. Nicholson, J.M. Gee, M.E. Harper, EGFR and cancer prognosis, Eur. J. Cancer 37 (2001) S9–S15.
[183] G.E. Kim, Y.B. Kim, N.H. Cho, H.C. Chung, H.R. Pyo, J.D. Lee, T.K. Park, W.S. Koom, M. Chun, C.O. Suh, Synchronous coexpression of epidermal growth factor receptor and cyclooxygenase-2 in carcinomas of the uterine cervix: a potential predictor of poor survival, Clin. Cancer Res. 10 (2004) 1366–1374.
[184] S. Movva, L. Rodriguez, H. Arias-Pulido, C. Verschraegen, Novel chemotherapy approaches for cervical cancer, Cancer 115 (2009) 3166–3180.
[185] T. Soonthornthum, H. Arias-Pulido, N. Joste, L. Lomo, C. Muller, T. Rutledge, C. Verschraegen, Epidermal growth factor receptor as a biomarker for cervical cancer, Ann. Oncol. 22 (2011) 2166–2178.
[186] R. Balan, N. Simion, S.E. Giuşcă, A. Grigoraş, L. Gheucă-Solovăstru, V. Gheorghiţă, C. Amălinei, I.D. Căruntu, Immunohistochemical assessment of p16, COX-2 and EGFR in HPV-positive cervical squamous intraepithelial lesions, Rom. J. Morphol. Embryol. 52 (2011) 1187–1194.
[187] G. Scambia, G. Ferrandina, M. Distefano, G. D'Agostino, P. Benedetti-Panici, S. Mancuso, Epidermal growth factor receptor (EGFR) is not related to the prognosis of cervical cancer, Cancer Lett. 123 (1998) 135–139.
[188] H.Y. Ngan, A.N. Cheung, S.S. Liu, D.K. Cheng, T.Y. Ng, L.C. Wong, Abnormal expression of epidermal growth factor receptor and c-erbB2 in squamous cell carcinoma of the cervix: correlation with human papillomavirus and prognosis, Tumour Biol. 22 (2001) 176–183.
[189] G.S. Akerman, W.H. Tolleson, K.L. Brown, L.L. Zyzak, E. Mourateva, T.S. Engin, A. Basaraba, A.L. Coker, K.E. Creek, L. Pirisi, Human papillomavirus type 16 E6 and E7 cooperate to increase epidermal growth factor receptor (EGFR) mRNA levels, overcoming mechanisms by which excessive EGFR signaling shortens the life span of normal human keratinocytes, Cancer Res. 61 (2001) 3837–3843.
[190] M. Schrevel, A. Gorter, S.M. Kolkman-Uljee, J.B. Trimbos, G.J. Fleuren, E.S. Jordanova, Molecular mechanisms of epidermal growth factor receptor overexpression in patients with cervical cancer, Mod. Pathol. 4 (2011) 720–728.
[191] A. Venuti, D. Salani, V. Manni, F. Poggiali, A. Bagnato, Expression of endothelin 1 and endothelin A receptor in HPV-associated cervical carcinoma: new potential targets for anticancer therapy, FASEB J. 14 (2000) 2277–2283.
[192] A. Bagnato, A. Cirilli, D. Salani, P. Simeone, A. Muller, M.R. Nicotra, P.G. Natali, A. Venuti, Growth inhibition of cervix carcinoma cells in vivo by endothelin A receptor blockade, Cancer Res. 62 (2002) 6381–6384.
[193] S. Singhal, A. Vachani, D. Antin-Ozerkis, L.R. Kaiser, S.M. Albelda, Prognostic implications of cell cycle, apoptosis, and angiogenesis biomarkers in non-small cell lung cancer: a review, Clin. Cancer Res. 11 (2005) 3974–3986.
[194] A.L. Gartel, The conflicting roles of the cdk inhibitor p21(CIP1/WAF1) in apoptosis, Leuk. Res. 29 (2005) 1237–1238.
[195] B.A. Werness, H.Q. Wang, J. Chance, D.J. Goldstein, p53-independent expression of p21waf1/cip1 in preinvasive and invasive squamous neoplasms of the uterine cervix, Mod. Pathol. 10 (1997) 578–584.
[196] X. Lu, T. Toki, I. Konishi, T. Nikaido, S. Fujii, Expression of p21WAF1/CIP1 in adenocarcinoma of the uterine cervix: a possible immunohistochemical marker of a favorable prognosis, Cancer 82 (1998) 2409–2417.
[197] H. Skomedal, G.B. Kristensen, A.K. Lie, R. Holm, Aberrant expression of the cell cycle associated proteins TP53, MDM2, p21, p27, cdk4, cyclin D1, RB, and EGFR in cervical carcinomas, Gynecol. Oncol. 73 (1999) 223–228.
[198] A.K. Lie, S. Skarsvåg, H. Skomedal, O.A. Haugen, R. Holm, Expression of p53, MDM2, and p21 proteins in high-grade cervical intraepithelial neoplasia and relationship to human papillomavirus infection, Int. J. Gynecol. Pathol. 18 (1999) 5–11.
[199] L.W. Huang, Y.Y. Chou, S.L. Chao, T.J. Chen, T.T. Lee, p53 and p21 expression in precancerous lesions and carcinomas of the uterine cervix: overexpression of p53 predicts poor disease outcome, Gynecol. Oncol. 83 (2001) 348–354.
[200] A. Giannoudis, C.S. Herrington, Differential expression of p53 and p21 in low grade cervical squamous intraepithelial lesions infected with low, intermediate, and high risk human papillomaviruses, Cancer 89 (2000) 1300–1307.
[201] L.W. Huang, S.L. Chao, J.L. Hwang, Y.Y. Chou, Down-regulation of p27 is associated with malignant transformation and aggressive phenotype of cervical neoplasms, Gynecol. Oncol. 85 (2002) 524–528.
[202] A. Sgambato, G.F. Zannoni, B. Faraglia, A. Camerini, E. Tarquini, D. Spada, A. Cittadini, Decreased expression of the CDK inhibitor p27Kip1 and increased oxidative DNA damage in the multistep process of cervical carcinogenesis, Gynecol. Oncol. 92 (2004) 776–783.
[203] J. Bouda, O. Hes, M. Koprivova, M. Pesek, T. Svoboda, L. Boudova, P27 as a prognostic factor of early cervical carcinoma, Int. J. Gynecol. Cancer 23 (2013) 164–169.
[204] A. Greenhough, H.J. Smartt, A.E. Moore, H.R. Roberts, A.C. Williams, C. Paraskeva, A. Kaidi, The COX-2/PGE2 pathway: key roles in the hallmarks of cancer and adaptation to the tumour microenvironment, Carcinogenesis 30 (2009) 77–86.
[205] S. Kulkarni, J.S. Rader, F. Zhang, H. Liapis, A.T. Koki, J.L. Masferrer, K. Subbaramaiah, A.J. Dannenberg, Cyclooxy-genase-2 is overexpressed in human cervical cancer, Clin. Cancer Res. 7 (2001) 429–434.
[206] J.Y. Kim, S.J. Lim, K. Park, C.M. Lee, J. Kim, Cyclooxygenase-2 and c-erbB-2 expression in uterine cervical neoplasm assessed using tissue microarrays, Gynecol. Oncol. 97 (2005) 337–341.
[207] J.H. Farley, V. Truong, E. Goo, C. Uyehara, C. Belnap, W.I. Larsen, A randomized double-blind placebo-controlled phase II trial of the cyclooxygenase-2 inhibitor Celecoxib in the treatment of cervical dysplasia, Gynecol. Oncol. 103 (2006) 425–430.
[208] L.A. Hefler, C. Grimm, P. Speiser, G. Sliutz, A. Reinthaller, The cyclooxygenase-2 inhibitor rofecoxib (Vioxx) in the treatment of cervical dysplasia grade II–III phase II trial, Eur. J. Obstet. Gynecol. Reprod. Biol. 125 (2006) 251–254.
[209] K. Kim, Y.T. Jeon, I.A. Park, J.W. Kim, N.H. Park, S.B. Kang, H.P. Lee, Y.S. Song, Cyclooxygenase-2 expression in cervical intraepithelial neoplasia, Ann. N. Y. Acad. Sci. 1171 (2009) 111–115.
[210] D.K. Gaffney, J. Holden, M. Davis, K. Zempolich, K.J. Murphy, M. Dodson, Elevated cyclooxygenase-2 expression correlates with diminished survival in carcinoma of the cervix treated with radiotherapy, Int. J. Radiat. Oncol. Biol. Phys. 49 (2001) 1213–1217.
[211] G. Ferrandina, L. Lauriola, M.G. Distefano, G.F. Zannoni, M. Gessi, F. Legge, N. Maggiano, S. Mancuso, A. Capelli, G. Scambia, F.O. Ranelletti, Increased cyclooxygenase-2 expression is associated with chemotherapy resistance and poor survival in cervical cancer patients, J. Clin. Oncol. 20 (2002) 973–981.
[212] J.S. Saldivar, D. Lopez, R.A. Feldman, R. Tharappel-Jacob, A. de la Rosa, D. Terreros, W.S. Baldwin, COX-2 overexpression as a biomarker of early cervical carcinogenesis: a pilot study, Gynecol. Oncol. 107 (2007) S155–S162.
[213] M. Huang, Q. Chen, J. Xiao, C. Liu, X. Zhao, Prognostic significance of cyclooxygenase-2 in cervical cancer: a meta-analysis, Int. J. Cancer 132 (2013) 363–373.
[214] P.J. Paley, K.A. Staskus, K. Gebhard, D. Mohanraj, L.B. Twiggs, L.F. Carson, S. Ramakrishnan, Vascular endothelial growth factor expression in early stage ovarian carcinoma, Cancer 80 (1997) 98–106.
[215] S.I. Ishigami, S. Arii, M. Furutani, M. Niwano, T. Harada, M. Mizumoto, A. Mori, H. Onodera, M. Imamura, Predictive value of vascular endothelial growth factor (VEGF) in metastasis and prognosis of human colorectal cancer, Br. J. Cancer 78 (1998) 1379–1384.
[216] Y. Ohta, Y. Tomita, M. Oda, S. Watanabe, S. Murakami, Y. Watanabe, Tumor angiogenesis and recurrence in stage I non-small cell lung cancer, Ann. Thorac. Surg. 68 (1999) 1034–1038.
[217] W.F. Cheng, C.A. Chen, C.N. Lee, L.H. Wei, F.J. Hsieh, C.Y. Hsieh, Vascular endothelial growth factor and prognosis of cervical carcinoma, Obstet. Gynecol. 96 (2000) 721–726.
[218] M.H. Kim, S.S. Seo, Y.S. Song, D.H. Kang, I.A. Park, S.B. Kang, H.P. Lee, Expression of cyclooxygenase-1 and-2 associated with expression of VEGF in primary cervical cancer and at metastatic lymph nodes, Gynecol. Oncol. 90 (2003) 83–90.
[219] P.O. Van Trappen, D. Steele, D.G. Lowe, S. Baithun, N. Beasley, W. Thiele, H. Weich, J. Krishnan, J.H. Shepherd, M.S. Pepper, D.G. Jackson, J.P. Sleeman, I.J. Jacobs, Expression of vascular endothelial growth factor (VEGF)–C and VEGF-D, and their receptor VEGFR-3, during different stages of cervical carcinogenesis, J. Pathol. 201 (2003) 544–554.
[220] J.O. Kang, S.E. Hong, The prognostic effect of VEGF expression in squamous cell carcinoma of the cervix treated with radiation therapy alone, J. Korean Med. Sci. 192 (2004) 693–697.
[221] Y. Dai, X. Zhang, Y. Peng, Z. Wang, The expression of cyclooxygenase-2, VEGF and PGs in CIN and cervical carcinoma, Gynecol. Oncol. 97 (2005) 96–103.
[222] K. Miller, M. Wang, J. Gralow, M. Dickler, M. Cobleigh, E.A. Perez, T. Shenkier, D. Cella, N.E. Davidson, Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer, N. Engl. J. Med. 357 (2007) 2666–2676.
[223] A. Sandler, R. Gray, M.C. Perry, J. Brahmer, J.H. Schiller, A. Dowlati, R. Lilenbaum, D.H. Johnson, Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer, N. Engl. J. Med. 355 (2006) 2542–2550.
[224] M. Branca, C. Giorgi, D. Santini, L. Di Bonito, M. Ciotti, A. Benedetto, P. Paba, S. Costa, D. Bonifacio, P. Di Bonito, L. Accardi, C. Favalli, K. Syrjänen, HPV-Pathogen ISS Study Group, Aberrant expression of VEGF-C is related to grade of cervical intraepithelial neoplasia (CIN) and high risk HPV, but does not predict virus clearance after treatment of CIN or prognosis of cervical cancer, J. Clin. Pathol. 59 (2006) 40–47.
[225] A. Gadducci, M.E. Guerrieri, C. Greco, Tissue biomarkers as prognostic variables of cervical cancer, Crit. Rev. Oncol. Hematol. 86 (2013) 104–129.
[226] F. Zagouri, T.N. Sergentanis, D. Chrysikos, M. Filipits, R. Bartsch, Molecularly targeted therapies in cervical cancer. A systematic review, Gynecol. Oncol. 126 (2012) 291–303.
[227] F.A. Suprynowicz, G.L. Disbrow, E. Krawczyk, V. Simic, K. Lantzky, R. Schlegel, HPV-16 E5 oncoprotein upregulates lipid raft components caveolin-1 and ganglioside GM1 at the plasma membrane of cervical cells, Oncogene 27 (2008) 1071–1078.
[228] J. Sun, J. Gao, J.B. Hu, L.F. Fan, X.B. Zhu, R. Subahan, H.L. Chen, Expression of Cav-1 in tumour cells, rather than in stromal tissue, may promote cervical squamous cell carcinoma proliferation, and correlates with high-risk HPV infection, Oncol. Rep. 27 (2012) 1733–1740.
[229] W.O. Lui, N. Pourmand, B.K. Patterson, A. Fire, Patterns of known and novel small RNAs in human cervical cancer, Cancer Res. 67 (2007) 6031–6043.
[230] X. Hu, J.K. Schwarz, J.S. Lewis Jr., P.C. Huettner, J.S. Rader, J.O. Deasy, P.W. Grigsby, X. Wang, A microRNA expression signature for cervical cancer prognosis, Cancer Res. 70 (2010) 441–448.
[231] W.C. McBee, A.S. Gardiner, R.P. Edwards, J.L. Lesnock, R. Bhargava, R.M. Austin, R.S. Guido, S.A. Khan, MicroRNA Analysis in Human Papillomavirus (HPV)-Associated Cervical Neoplasia and Cancer, J. Carcinog. Mutagen. 5 (1) (2011) 114.
[232] G. Deftereos, S.R. Corrie, Q. Feng, J. Morihara, J. Stern, S.E. Hawes, N.B. Kiviat, Expression of mir-21 and mir-143 in cervical specimens ranging from histologically normal through to invasive cervical cancer, PLoS One 6 (2011) e28423.
[233] L. Liu, X. Yu, X. Guo, Z. Tian, M. Su, Y. Long, C. Huang, F. Zhou, M. Liu, X. Wu, X. Wang, miR-143 is downregulated in cervical cancer and promotes apoptosis and inhibits tumor formation by targeting Bcl-2, Mol. Med. Rep. 5 (2012) 753–760.
[234] F. Vasilescu, M. Ceauşu, C. Tănase, R. Stănculescu, T. Vlădescu, Z. Ceauşu, P53, p63 and Ki-67 assessment in HPV-induced cervical neoplasia, Rom. J. Morphol. Embryol. 50 (2009) 357–361.
[235] A.N. Cheung, K.L. Tsun, K.M. Ng, E. Szeto, M.K. Siu, E.S. Wong, H.Y. Ngan, P634A4 and TAp73 immunocytochemistry in liquid-based cervical cytology—potential biomarkers for diagnosis and progress prediction of cervical neoplasia, Mod. Pathol. 23 (2010) 559–566.
[236] Y.F. Wong, T.K. Chung, V.W. Wang, D.I. Smith, DNA microchips to identify molecular signatures in cervical cancers, Methods Mol. Biol. 385 (2007) 87–101.

[237] A. Szalmas, J. Konya, Epigenetic alterations in cervical carcinogenesis, Semin. Cancer Biol. 19 (2009) 144–152.
[238] H.J. Yang, V.W. Liu, Y. Wang, P.C. Tsang, H.Y. Ngan, Differential DNA methylation profiles in gynecological cancers and correlation with clinico-pathological data, BMC Cancer 6 (2006) 1–10.
[239] N. Wentzensen, M. von Knebel Doeberitz, Biomarkers in cervical cancer screening, Dis. Markers 23 (2007) 315–330.
[240] H.J. Yang, Aberrant DNA methylation in cervical carcinogenesis, Chin. J. Cancer 32 (2013) 42–48.
[241] C.J. Lin, H.C. Lai, K.H. Wang, C.A. Hsiung, H.W. Liu, D.C. Ding, C.Y. Hsieh, T.Y. Chu, Testing for methylated PCDH10 or WT1 is superior to the HPV test in detecting severe neoplasms (CIN3 or greater) in the triage of ASCUS smear results, Am. J. Obstet. Gynecol. 204 (2011) 21.e1–21.e7.
[242] T.K. Chao, F.Y. Ke, Y.P. Liao, H.C. Wang, C.P. Yu, H.C. Lai, Triage of cervical cytological diagnoses of atypical squamous cells by DNA methylation of paired boxed gene 1 (PAX1), Diagn. Cytopathol. 41 (2011) 41–46.
[243] J.J. Eijsink, N. Yang, A. Lendvai, H.G. Klip, H.H. Volders, H.J. Buikema, B.M. van Hemel, M. Voll, H.J. Coelingh Bennink, E. Schuuring, G.B. Wisman, A.G. van der Zee, Detection of cervical neoplasia by DNA methylation analysis in cervico vaginal lavages, a feasibility study, Gynecol. Oncol. 120 (2011) 280–283.
[244] R.M. Overmeer, J.A. Louwers, C.J. Meijer, F.J. van Kemenade, A.T. Hesselink, N.F. Daalmeijer, S.M. Wilting, D.A. Heideman, R.H. Verheijen, A. Zaal, W.M. van Baal, J. Berkhof, P.J. Snijders, R.D. Steenbergen, Combined CADM1 and MAL promoter methylation analysis to detect (pre) malignant cervical lesions in high risk HPV positive women, Int. J. Cancer 129 (2011) 2218–2225.
[245] A. Botezatu, C.D. Goia-Rusanu, I.V. Iancu, I. Huica, A. Plesa, D. Socolov, C. Ungureanu, G. Anton, Quantitative analysis of the relationship between microRNA-124a, -34b and-203 gene methylation and cervical oncogenesis, Mol. Med. Rep. 4 (2011) 121–128.
[246] K. Heselmeyer, E. Schrock, S. du Manoir, H. Blegen, K. Shah, R. Steinbeck, G. Auer, T. Ried, Gain of chromosome 3q defines the transition from severe dysplasia to invasive carcinoma of the uterine cervix, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 479–484.
[247] M. Lando, S.M. Wilting, K. Snipstad, T. Clancy, M. Bierkens, E.K. Aarnes, M. Holden, T. Stokke, K. Sundfør, R. Holm, G.B. Kristensen, R.D. Steenbergen, H. Lyng, Identification of eight candidate target genes of the recurrent 3p12-p14 loss in cervical cancer by integrative genomic profiling, J. Pathol. 230 (2013) 59–69.
[248] V.N. Senchenko, N.P. Kisseljova, T.A. Ivanova, A.A. Dmitriev, G.S. Krasnov, A.V. Kudryavtseva, G.V. Panasenko, E.B. Tsitrin, M.I. Lerman, F.L. Kisseljov, V.I. Kashuba, E.R. Zabarovsky, Novel tumor suppressor candidates on chromosome 3 revealed by NotI-microarrays in cervical cancer, Epigenetics 8 (2013) 59–69.
[249] K. Heselmeyer-Haddad, K. Sommerfeld, N.M. White, N. Chaudhri, L.E. Morrison, N. Palanisamy, Z.Y. Wang, G. Auer, W. Steinberg, T. Ried, Genomic amplification of the human telomerase gene (TERC) in pap smears predicts the development of cervical cancer, Am. J. Pathol. 166 (2005) 1229–1238.
[250] S. Andersson, P. Sowjanya, D. Wangsa, A. Hjerpe, B. Johansson, G. Auer, P.E. Gravitt, C. Larsson, K.L. Wallin, T. Ried, K. Heselmeyer-Haddad, Detection of genomic amplification of the human telomerase gene TERC, a potential marker for triage of women with HPV-positive, abnormal Pap smears, Am. J. Pathol. 175 (2009) 1831–1847.
[251] G.R. Jalali, T.J. Herzog, B. Dziura, R. Walat, M.W. Kilpatrick, Amplification of the chromosome 3q26 region shows high negative predictive value for nonmalignant transformation of LSIL cytologic finding, Am. J. Obstet. Gynecol. 202 (2010) 581.e1–581.e5.
[252] A. Ziegler, A. Koch, K. Krockenberger, A. Grosshennig, Personalized medicine using DNA biomarkers: a review, Hum. Genet. 131 (2012) 1627–1638.
[253] A.C. Freitas, A.P. Gurgel, B.S. Chagas, E.C. Coimbra, C.M. do Amaral, Susceptibility to cervical cancer: an overview, Gynecol. Oncol. 126 (2012) 304–311.
[254] B.S. Chagas, M.V. Batista, V. Guimarães, V.Q. Balbino, S. Crovella, A.C. Freitas, New variants of E6 and E7 oncogenes of human papillomavirus type 31 identified in Northeastern Brazil, Gynecol. Oncol. 123 (2011) 284–288.
[255] B.S. Chagas, M.V. Batista, S. Crovella, A.P. Gurgel, J.D. Silva Neto, I.G. Serra, C.M. Amaral, V.Q. Balbino, M.T. Muniz, A.C. Freitas, Novel E6 and E7 oncogenes variants of Human Papillomavirus Type 31 in Brazilian Women with Abnormal Cervical Cytology, Infect. Genet. Evol. 10 (2013) 13–18.