 doi:10.20944/preprints202004.0294.v1

Signaling Pathways in Cervical Cancer Chemoresistance: Are microRNAs and Long-Noncoding RNAs the Main Culprits?

Seyyed Reza Mousavi[a, b], Nima Hemmat[a], Hossein Bannazadeh Baghi[a, c], Afshin Derakhshani[a], Stefania Tommasi[d], Oronzo Brunetti[e], Behzad Baradaran*[a, f], Nicola Silvestris*[e, g]

a) Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
b) Department of Medical Genetics, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.
c) Department of Virology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
d) Molecular Diagnostics and Pharmacogenetics Unit, IRCCS Istituto Tumori di Bari "Giovanni Paolo II" - Bari
e) Medical Oncology Unit, IRCCS Istituto Tumori di Bari "Giovanni Paolo II" - Bari
f) Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
g) Department of Biomedical Sciences and Human Oncology, DIMO - University of Bari, Italy

*Corresponding Authors:

Correspondence: n.silvestris@oncologico.bari.it; Tel.: +39 3332228492 (N.S.).

baradaranb@tbzmed.ac.ir: Tel: +98 413 3371440 (B.B.)

 doi:10.20944/preprints202004.0294.v1

Abstract

Cervical cancer is known as one of the most important cancers in women worldwide. Chemotherapy is a standard treatment for advanced/recurrent cervical cancer in which the prognosis of the disease is really poor and the 1-year survival chance in these patients is maximally 20%. However, resistance to anticancer drugs is a major problem in treating cancer. Cervical cancer stem cells are considered as a fundamental cause of chemo and radio-resistance and also relapse after primary successful treatment. Signaling pathways include a wide range of molecular mechanisms contribute to drug resistance. Recently, microRNAs (miRNAs) are announced as a group of molecular biomarkers involving in response to chemotherapy in cancer patients. As the miRNAs, there are some long non-coding RNAs (LncRNAs) which their aberrant expression is considered as a biomarker for monitoring chemoresistance. In this review, we summarized current reports about the involvement of signaling pathways during chemoresistance in cervical cancer. Then, genes that have been demonstrated their involvement during drug resistance in cervical cancer were tabulated. Further, miRNAs that have been reported as biomarkers during treatment are listed. By bioinformatic analysis, we predictedmiR-335-5p and miR-16-5p as the most potential biomarkers for monitoring resistance to chemotherapy. Finally, long non-coding RNAs that have been introduced in recent studies as novel biomarkers during the response to chemotherapy were mentioned.

Keywords: signaling pathway, microRNA, long-noncoding RNA, chemoresistance, cervical cancer

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 April 2020 doi:10.20944/preprints202004.0294.v1

Introduction

Cervical cancer (CC) is known as one of the most important cancers in females. Yearly, several new cases of this cancer are reported to the World Health Organization (WHO), which makes this cancer a global issue[1]. There are several risk factors, which could trigger or deteriorate the incidence of CC including gene polymorphisms, multiple sexual partners, lamin A/C deficiency, and smoking [2-6]. Actually, it has been demonstrated that the main cause of this cancer is Human papillomavirus (HPV) infection[7]. The importance of this infection is highlighted as its ability to be the initiator of approximately 5% of all human cancers[8]. Among the encoded proteins of HPV, its oncoproteins (E5, E6, and E7) are the principle actors in the pathogenesis and carcinogenesis of this virus[9, 10]. As the HPV infection leads to CC, the initial action in the way of its treatment is chemotherapy[11].

Chemotherapy is a standard treatment for advanced/recurrent cervical cancer in which the prognosis of the disease is really poor and the 1-year survival chance in these patients is maximally 20%[12].Some chemo drugs kill the cancerous cells by targeting and damaging their DNA. Currently, many drugs are developed to block the growth and proliferation of cancer cells. However, despite these achievements in chemotherapy, resistance to anticancer drugs is a remarkable problem in treating cancer. Various cellular and molecular mechanisms including genetics and epigenetics alternation, excessive drug efflux, and decreased accumulation of drug have been reported to contribute to the chemoresistance of cancers. Overall, based on the progression time, drug resistance can be divided into inherent and acquired groups. In the inherent form, drug resistance is present before the exposure of cancer cells to anti-cancer drugs. This type of resistance could be the result of some mutations in

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 April 2020 doi:10.20944/preprints202004.0294.v1

genes involved in cancer cell proliferation and apoptosis. Acquired resistance develops after the first treatment with anticancer drugs which can be due to the altered expression level of drug targets. Therefore, understanding the exact molecular mechanisms underlying chemoresistance in CC patients can help to investigate better therapeutic approaches based on the patient's susceptibility[12, 13]. Recently, microRNAs (miRNAs) are considered as a novel group of molecular biomarkers for monitoring the response to chemotherapy in cancer patients. miRNAs are a group of non-coding RNAs with the 21-23 nucleotides length, which post-transcriptionally regulate the expression of their targets [14]. As the involvement of miRNAs in the vital biological processes including cell cycle, they can play important roles in the development of malignancies and drug resistance by targeting some important signaling pathways [15]. Long noncoding RNAs (LncRNAs) are a group of RNAs longer than 200 nucleotides in length lacking significant open reading frames [16]. Like miRNAs, lncRNAs have vital roles in cellular processes such as transcriptional and post-transcriptional modifications and disruption in their functions may result in disease conditions particularly cancer. Recently, it has been reported lncRNAs could play both oncogenic or tumor suppressing activity during cancer development and also mediate drug resistance/sensitivity in cancer cells [17, 18].

Some in vitro and in vivo studies have reported the dysregulation of various miRNAs and lncRNAs during chemoresistance in the CC cells and patients. In this study, we reviewed the, signaling pathways, miRNAs, and lncRNAs that are involved in drug resistance in CC.

Role of signaling pathways in chemoresistance

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 April 2020 doi:10.20944/preprints202004.0294.v1

Signaling pathways include a wide range of molecular mechanisms that contribute to drug resistance. In the following parts, a list of some reported signaling pathways underlying drug resistance in CC has been provided (Figure 1).

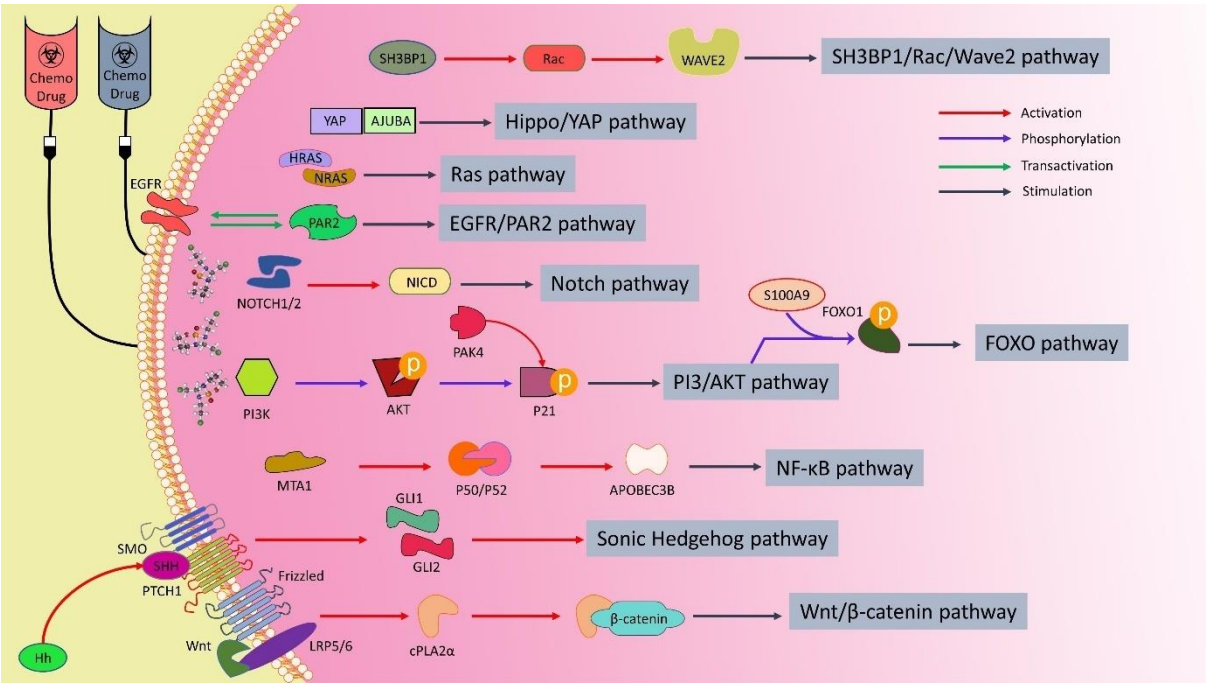

Figure 1: Signaling pathways involved in the chemoresistance of CC

Wnt/β-catenin signaling pathway

The Wnt signaling is a highly conserved pathway involved in vital processes including differentiation and proliferation. It is known that ectopic activation of this pathway or genetic abnormality in Wnt signaling components could lead tovarious cancers, including CC. Among these components, Wnt ligands have the most contribution to developing cancer.To prove this claim, the deregulation of Wnt7A and Wnt14 has been reported in CCcells and biopsies derived from CC patients. cPLA2α is one of the isoforms of Phospholipases A2s

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 April 2020 doi:10.20944/preprints202004.0294.v1

(PLA2s) that convert phospholipids to arachidonic acid in order to produce lipid mediators including prostaglandins. Hai Xu, *et al*. showed the overexpression of cPLA2α in patients with CC in comparison with low to moderate expression in normal cervical tissues. Moreover, it has been illustrated that cPLA2α affects the response to chemotherapy in cancer cells. In other words, blockage of cPLA2α makes cancer cells sensitive to chemotherapy and get a better response to therapy. β-catenin, which is a key mediator in the Wnt signaling pathway, is determined as a downstream target of cPLA2α. The role of Wnt/β-catenin in the progression of tumor invasion also has been reported. It has been depicted that inhibition of cPLA2α results in β-catenin suppression in cancer stem cells. Therefore, the suppression of Wnt/β-catenin through cPLA2α inhibition could lead to chemosensitivity[19-21].

Sonic Hedgehog signaling pathway

Newly, the involvement of the Hedgehog (Hh) signaling pathway in the tumorigenicity in bladder cancer has been determined[22].The mRNA expression level of some Hh signaling pathway members including, *PTCH1*, *SMO*,*GLI1*, and *GLI2*wasupregulated in epithelial to mesenchymal transition (EMT)-induced CC cells compared with control cells. Thus, it was hypothesized that the inhibition of the Hh pathway could improve the response to therapy in CC patients. During EMT, the expression level of E cadherins expressively decreased. Gli-antagonist GANT58 is one of the Hh signaling pathway inhibitors. The combination of GANT58 upregulation with cisplatin decreases viability and invasiveness in E-cadherin low CC cell lines and leads to a better response to treatment[23].

NF-kB signaling pathway

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 April 2020 doi:10.20944/preprints202004.0294.v1

The NF-kB family includes transcription factors that are involved in the initiation and progression of various cancers. It has been shown that activation of the NF-kB signaling was significantly induced in cancerous cell lines exposed to cytotoxic agents. Moreover, the high expression level of NF-kB may contribute to poor response to treatment with cisplatin in cancer cell lines [24]. In CC, the association of NF-kB signaling with chemoresistance has been investigated. Moreover, up-regulated levels of Metastasis-Associated Protein 1 (MTA1) and APOBEC3B have been reported in *in vitro* experiments evaluating CDDP resistance in cancer cells from cervical tumors. MTA1 inversely regulates APOBEC3B throughout the NF-kB signaling in HPV infected cells. Furthermore, NF-kB inhibition decreased expression of APOBEC3B and knockdown of MTA1 re-sensibilized metastatic cancer cells of cervical tumor to platinum salt therapy [25].

Phosphatidylinositol 3-kinase and protein kinase B signaling pathway

The Phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) is very important pathway implicated in biological processes such as cell proliferation and anti-apoptosis. Several physiological stimuli such as hormones and/or growth factor improve extracellular signaling and lead to the activation of PI3K signaling. It has been known that PI3K/Akt contributes to the development of CC [26]. Zhang *et al*. showed that the PI3K levels were significantly induced in cancer tissues compared with the adjacent normal tissue of cervical tumor [27].

In recent studies, it has been shown that PI3K/Akt signaling directly or indirectly is involved in chemoresistance. p21-activated kinases (PAKs) are a group of serine/threonine protein kinases that contribute to tumorigenesis by regulating cell cycle progression. The overexpression of PAK4 in tumor cervical tissues in comparison to pretumor tissues has been reported. It has been shown that the upregulation of the PAK4 alleviates the response rate to cisplatin therapy in a PI3K/Akt dependent way. Moreover, the knockout of PAK4 leads to

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 April 2020 doi:10.20944/preprints202004.0294.v1

better response to cisplatin through the reduction of the cell viability in Hela and Caski cells. The phosphorylated form of Akt was significantly increased after cisplatin treatment. But the promotion of phosphorylated Akt was inhibited after the suppression of PAK4.Furthermore, it was determined that LY294002, a PI3K/Akt inhibitor sensitizes the Hela and Caski cells to cisplatin therapy[28].

Notch signaling pathway

The Notch signaling pathway is also propounded to contribute to carcinogenesis. Notch1, as a component of the Notch signaling pathway, plays an important role in the initiation and progression of various tumors. In different cancers including, colon cancer and brain cancer, the oncogenic role of Notch signaling has been reported[29]. Previous studies have demonstrated that Notch signaling can act as either oncogene or tumor suppressor in CC. Li Sun1 *et al*. showed that the expression level of Notch1 was extensively decreased during the development of normal cervical epithelium in comparison with cervical intraepithelial neoplasia (CIN). The reduced activity of Notch1 leads to the enhanced expression of HPV E6 and E7 genes in the HPV-infected cervical epithelium cells [30]. On the other hand, tumor metastasis and invasiveness were identified in CC patients with the high expression level of Notch1/JAG1[31]. Wang, L., *et al.* determined that the expression level of notch2 was significantly increased in CC cells compared with the normal manipulated cervical cells. Further, the inhibition of the Notch signaling pathway via γ-secretase inhibitor (GSI) RO4929097reduces the chemoresistance and metastasis in CC cells [31].

Forkhead box O (FOXO) signaling pathway

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 April 2020 doi:10.20944/preprints202004.0294.v1

FOXO term is appointed to a group of transcription factors involved in various cellular processes such as apoptosis, proliferation and DNA repair. The contribution of the FOXO pathway in carcinogenesis has been proposed as well. Due to the anti-proliferative and pro-apoptosis feature of FOXO, itis suggested that FOXO could play an important role as a tumor suppressor in various cancers [32]. There are several studies that have focused on the altered expression level of FOXO proteins in CC. B. Zhang *et al*. showed that the expression level of FOXO1 protein was significantly decreased in the HPV-positive cell lines (HeLa, Caski, SiHa) compared with HPV-negative cell line (C-33A). Furthermore, it has been reported that the overexpression of FOXO1 leads to the upregulation of caspase-3 and caspase-9 in SiHa cells and therefore promotes apoptosis [33]. S100 calcium-binding protein A9 (S100A9) has been determined to be upregulated in diverse cancers and plays an important role in resistance to chemotherapy. The overexpression of S100A9 boosts the phosphorylation and inhibition of FOXO1 through PI3K/AKT and MEK/ERK signaling pathways and leads to poor response to cisplatin-treatment in SiHa cells [34]. FOXO1 directly regulates the apoptotic genes including *OCT4*, *SOX2*, and NANOG. Thus, the resistance to cisplatin-therapy could be the result of decreased apoptosis rate due to the FOXO1 phosphorylation [34].

EGFR and TF-PAR2 signaling pathways

Protease-activated receptors (PARs) are a group of transmembrane G-coupled receptors which are activated by cleavage in the extracellular domain. A link between proteolytic activity and the development of CC has been reported [34]. Sánchez-Hernández, *et al*. proved that the expression level PAR2 is significantly increased in CC cell lines and 16 patients specimens[35]. de Almeida *et al*. showed that PAR2 can transactivate the epidermal growth

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 April 2020 doi:10.20944/preprints202004.0294.v1

factor receptor (EGFR) [36]. EGFR is a transmembrane glycoprotein receptor that belongs to HER tyrosine- kinase receptor family. It has been shown that EGFR is upregulated in various solid tumors including CC. The HPV-E5 protein increases the EGFR expression and also inhibits its degradation[37, 38]. Moreover, EGFR stimulates tissue factor (TF) which could transactivate the PAR2 in a positive feedback loop. EGFR and PAR2 are involved in the response to cisplatin treatment. It has been reported that the activation of the PAR2-EGFR-TF signaling pathway decreases the induction of caspase3-cleavage by cisplatin and therefore leads to chemoresistance[36].

JAK-STAT signaling pathway

Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway is considered as a pivotal modulator of cellular processes including cell proliferation, migration, and apoptosis[39]. It has been determined that the JAK-STAT is involved in the initiation and development of several cancer types and plays an important role during the response to chemotherapy. L. HUANG1 *et al*. found that the expression level of STAT3 is significantly upregulated in CC cells compared with the normal cervical mucosa. Moreover, it was shown that in a patient with no noticeable response to chemotherapy, the expression of *STAT3* is higher in comparison with the patient's group who responded to chemotherapy obviously, which may prove the involvement of *STAT3* in chemoresistance [40].

RAS signaling

Ras is a proto-oncogene which belongs to the GTP-binding proteins and contributed to various biological processes [41]. The mutated or overexpressed form of RAS has been demonstrated to be involved in many cancers. There are some studies that have discussed the role of Ras signaling in HPV-induced carcinogenesis such as CC. It has been reported that the activation of Ras can lead to overexpression of HPV-E6 and E7 proteins [42]. N. Mammas *et*

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 April 2020 doi:10.20944/preprints202004.0294.v1

al. revealed that the expression level of the two 2 Ras oncogenes (H-Ras, N-Ras) was upregulated in CC cases in comparison with the normal cervical tissues [43]. The involvement of Ras signaling during the response to chemotherapy has been suggested. The two crucial pathways, PI3K/Akt/mTOR and Raf/MEK/ERK which are pivotal for the growth and survival of cancer cells are stimulated by Ras activation[44]. By using cell culture and mouse model, Xu J *et al*. showed that the activation of Ras was inhibited by zoledronic acid (ZA) in CC. Furthermore, the PI3K/Akt/mTOR and Raf/MEK/ERK were deactivated in CC cells exposed to ZA and therefore sanitize cancerous cells to chemotherapy [45].

Hippo/YAP signaling pathway

Hippo pathway is an important cell signaling involved in the control of organ size. Consistent with the result of several studies, the Hippo pathway is involved in the progression of CC. *YAP* protein is the main effector of the Hippo signaling pathway that its overexpression has been reported in CC tissues. The upregulation of *YAP* could promote the proliferation and migration through EGFR signaling in the CC cell lines. Moreover, HPV-E6 protein protects *YAP* protein from degradation and may lead to a consistent proliferation in CC[46].*A JUBA*, which is known as an oncogene, has been reported to be involved in the tumorigenesis by affecting cell proliferation and migration. Lihong Bi *et al*. found that the expression level of *AJUBA* was significantly increased in CC tissues in comparison with the adjacent tissues and leads to chemoresistance in CC[47].

SH3BP1/Rac/Wave2 signaling

Rac belongs to the GTPases family and regulates various cellular functions which result in the medullation of cell mortality and invasion. Ras also can effect on apoptosis and cell proliferation and thereby contribute to the tumorigenesis[48]. SH3domain-binding protein-1 (SH3BP1) can induce CC by stimulation of *Rac* and its target *Wave2*. The overexpression of

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 April 2020 doi:10.20944/preprints202004.0294.v1

SH3BP1 in CC tissues in comparison with normal tissues has been reported. The *SH3BP1* knockdown leads to the decreased invasion and migration in the Hela and Caski cells. Moreover, the *SH3BP1* overexpression increases the activation of the Rac and Wave2 in Hela and Caski cells, suggesting that *SH3BP1* may lead to CC through Rac/Wave2 signaling. Furthermore, the SH3BP1 overexpression exacerbates the response to cisplatin-based chemotherapy. However, SH3BP1 knockdown leads to a better response during cisplatin-based treatment [49].

Association of miRNAs to chemoresistance

Recently, miRNAs have been considered as diagnostic and prognostic biomarker for cancer treatment and researchers have focused on them as potentially therapeutic molecules [50].

Interestingly, various studies have discussed the role of miRNAs during the resistance to chemotherapy. The different expression level of some miRNAs in cancerous drug resistance cells compared with drug-sensitive cancerous cell has been reported[51]. However, the exact mechanism of action about dysregulated miRNAs in chemoresistance remains unknown, it is supposed that miRNAs can alter the expression level of some proteins which are involved in the response to various chemo drugs. This disturbance could be exemplified that overexpression of miRNAs might down-regulate the expression of the genes which are responsible for drug efficiency. In contrast, decreased expression of miRNAs can lead to the up-regulation of the multidrug resistance gens such as ABC transporters which disrupt drug function, or the genes which promote proliferation, prevent apoptosis, etc. Consistent with the tissue specificity regulation of miRNAs, the same miRNA can play different roles in the different cancerous cells [51-53]. In CC, some miRNAs have been reported which their dysregulation could contribute to the chemoresistance.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 April 2020 doi:10.20944/preprints202004.0294.v1

miR-217:

The role of miR-217 in the tumorigenicity of various cancers has been demonstrated. Zhaojun Yin *et al*. found that the expression level of miR-217 was significantly reduced in CC cells. Moreover, in order to investigate the impact of miR-217 on the proliferation in CC, the miR-217 mimic was transfected to the SiHa and Caski cells. It was determined that the upregulation of miR-217 considerably suppresses the growth of CC cells. Furthermore, the upregulation of miR-217 could reduce the resistance to cisplatin-based therapy in CC patients. Finally, *KRAS* was identified as the target of miR-217 in CC. It has been shown that the protein level of KRAS was significantly decreased in the SiHa and CaSki cells which were transfected by miR-217 mimics compared with prenatal cells[54].

miR-101:

miR-101 is involved in CC through regulating its target genes including *Fos*.miR-101represses the *Fos* expression and inhibits the cell cycle in G1 to S transition phase. In HeLa cells, the downregulation of miR-101 leads to overexpression of the *Fos* gene and therefore enhances the proliferation and increases the resistance to the chemo-radiotherapy[55].

miR-21

The upregulation of miR-21 in the CC tissues and cell lines has been demonstrated. *GAS5*, as a direct target of miR-21 acts as a tumor suppressor which promotes apoptosis and represses migration. In CC tissue, an increased in the expression of miR-21 could downregulate the *GAS5*. Additionally, it has been reported that high expression of *GAS5* enhances sensitivity to cisplatin and therefore could be used as a potential biomarker for in chemoresistance[56, 57].Further, polymorphism in the miR-21 gene (rs1292037 (A > G)) might enhance the chemoresistance to cisplatin in the CC [58].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 April 2020 doi:10.20944/preprints202004.0294.v1

miR-134-5p

The association among lncRNA NCK1-AS1, miR-134-5p, and MSH2 has been examined. It was proved that the expression level of miR-134-5p was significantly reduced in CC HeLa cells. By using siRNA against the NCK1-AS1, it was clarified that the knockdown of NCK1-AS1 may lead to the upregulation of miR-134-5p and downregulation of MSH2whichwherebydecreases the resistance to cisplatin-based chemotherapy [59].

miR-130a

It has been reported that the expression level of miR-130a is significantly increased in cisplatin -resistance CC tissues in comparison to DDP-sensitive tissues. Copper transporter protein 1 (CTR1), is a protein involved in the regulation of cisplatin uptake into the cells. Chenzhe Fenga, *et al*. found that the knockdown of *CTR1*aggravates the resistance to the cisplatin in the HeLa and Caski cells. Moreover, miR-130a inhibits the *CTR1* expression and thereby enhances the resistance to the cisplatin in the CC cells[60]. However, in the other study, it was reported that miR-130a has been downregulated in the cisplatin- CC cells [61].

miR-214

The tumor suppressor role of miR-214 in CC has been investigated. miR-214 can sensitize the CC cells to cisplatin by targeting the *Bcl2l2*. The overexpression of *Bcl2l2* which belongs to the Bcl2 family is involved in cell survival and chemoresistance. It has been shown that miR-214 represses the mRNA and protein expression level of *Bcl2* and thus contributes to chemosensitivity in CC cells [62]. Further, Mitochondrial transcription factor A (TFAM) is a protein which its overexpression in malignancy of CC has been proved. It can promote

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 April 2020 doi:10.20944/preprints202004.0294.v1

migration and proliferation in the HeLa and Caski cells. miR-214 targets *TFAM* and therefore makes susceptible the HeLa and Caski cells to the cisplatin-based chemotherapy[63].

miR-27a and miR-451

miR-27a and miR-451 are involved in the regulation of the MDR gene [64]. Multidrug resistance (MDR) is defined as the ability of cancerous cells to survive after exposure to chemotherapeutic drugs and which is the main cause for failure in cancer treatment. P-glycoprotein is the product of the MDR gene which able the cancerous cells to gain resistance to chemotherapeutic drugs [65]. It has been reported that the expression level of miR-27a and miR-451 is overexpressed in the MDR CC KBV1 cell line. By using antagomirs against these two miRNAs, the mRNA expression level of P-glycoprotein and MDR was significantly reduced. This reduction leads to a better response to chemotherapy in MDR cervical cells [64].

miR-20a

The upregulation of miR-20a promotes cell proliferation and invasion in CC. Ying Xiong1 *et al*. proved in the SiHa cells which were transfected by iASPP (Inhibitor of apoptosis-stimulating protein of p53), the expression of miR-20a was significantly increased. It was clarified that iASPP upregulates the miR-20a by releasing the p53 from the promoter of miR-20a. The high expression of miR-20a induces resistance to the cisplatin in CC cells[66].

miR-629

miR-629 directly targets the Ras suppressor-1 (RSU1). RSU1 induces apoptosis in response to 1′S-1′-acetoxychavicol acetate (ACA) in the Caski and HeLa cells. The overexpression of

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 April 2020 doi:10.20944/preprints202004.0294.v1

miR-629 decreases the expression of RSU1. NH Phuah *et al*. reported that suppression of miR-629 in both Caski and HeLa cells enhances better response to ACA[67].

miR-25-3p

miR-25-3p is involved in the regulation of the epithelial-mesenchymal transition (EMT)in cisplatin-resistance CC cells. This regulation is mediated by targeting the Sema4C gene. The upregulation of miR-25-3p or decreased expression of Sema4C could promote sensitivity to the cisplatin-based treatment and reversed the EMT phenotype in the CR HeLa and Caski cells[68].

miR-182, miR-30a

miR-182 directly targets the Programmed Cell Death Protein 4 (PDCD4) which is a pro-apoptotic protein and involved in cell proliferation. It was noticed that thigh expression of miR-182 downregulates PDCD4 in the CR HeLa cells. PDCD4 downregulation could reduce the apoptosis rate and contributes to the cisplatin-resistance. The expression level of miR-30a was decreased in the CR CC cell line, which is supposed that effects on the autophagy genes[69].

miR-125a

It has been reported that the downregulation of miR-125a leads to resistance to paclitaxel and cisplatin. One target of miR-125a is *STAT3*. The inhibition of apoptosis, as a function of *STAT3*, has been proposed [70]. Z Fan *et al*. showed that in the paclitaxel-resistant HeLa and Caski cells, the expression of *STAT3* was significantly induced. Thereby, it was supposed that miR-125a may induce apoptosis by downregulating the *STAT3* and therefore promotes paclitaxel and cisplatin sensitivity in CC[71].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 April 2020 doi:10.20944/preprints202004.0294.v1

miR-181a

Yiran Chen *et al*. showed the expression of miR-181a increases the chemoresistance to cisplatin. One of the miR-181a targets is *PRKCD*, which induces apoptosis. It has been proved that miR-181a negatively regulates *PRKCD* and leads to impair apoptosis in SiHa and Me180 cell lines[72]. However, in the other study, it was shown the downregulation of miR-181a leads to resistance the oxaliplatin-based chemotherapy. *GRP78* is a direct target of miR-181a which promotes cell proliferation and oxaliplatin resistance in the CC subcutaneous model. Consequently, it has been supposed that miR-181a could sensitize CC cells to the oxaliplatin by downregulating the GRP78[73].

In-silico analysis

By using the miRTarBase online database, all validated targets of the mentioned miRNAs were collected. It was elucidated *PTEN* can be the most significant target based on the number of interactions with the intended miRNAs. Eight miRNAs can interact with the *PTEN* gene as a direct target (Fig.2). The interaction of these miRNAs with *PTEN* in various cancer has been determined. *PTEN* is a tumor suppressor that regulates vital biological processes such as cell proliferation and migration. It reduces the proliferation by inhibiting the Akt signaling pathway. Lack of *PTEN* expression contributes to various cancers including CC[74]. Further, dysregulation of *PTEN* as the target of miRNAs during chemoresistance in CC has been reported. For instance, miR-130a promotes proliferation by targeting PTEN and increases resistance to chemotherapy[60]. However, further studies are needed to clarify the exact molecular mechanism of PTEN in chemoresistance.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 April 2020 doi:10.20944/preprints202004.0294.v1

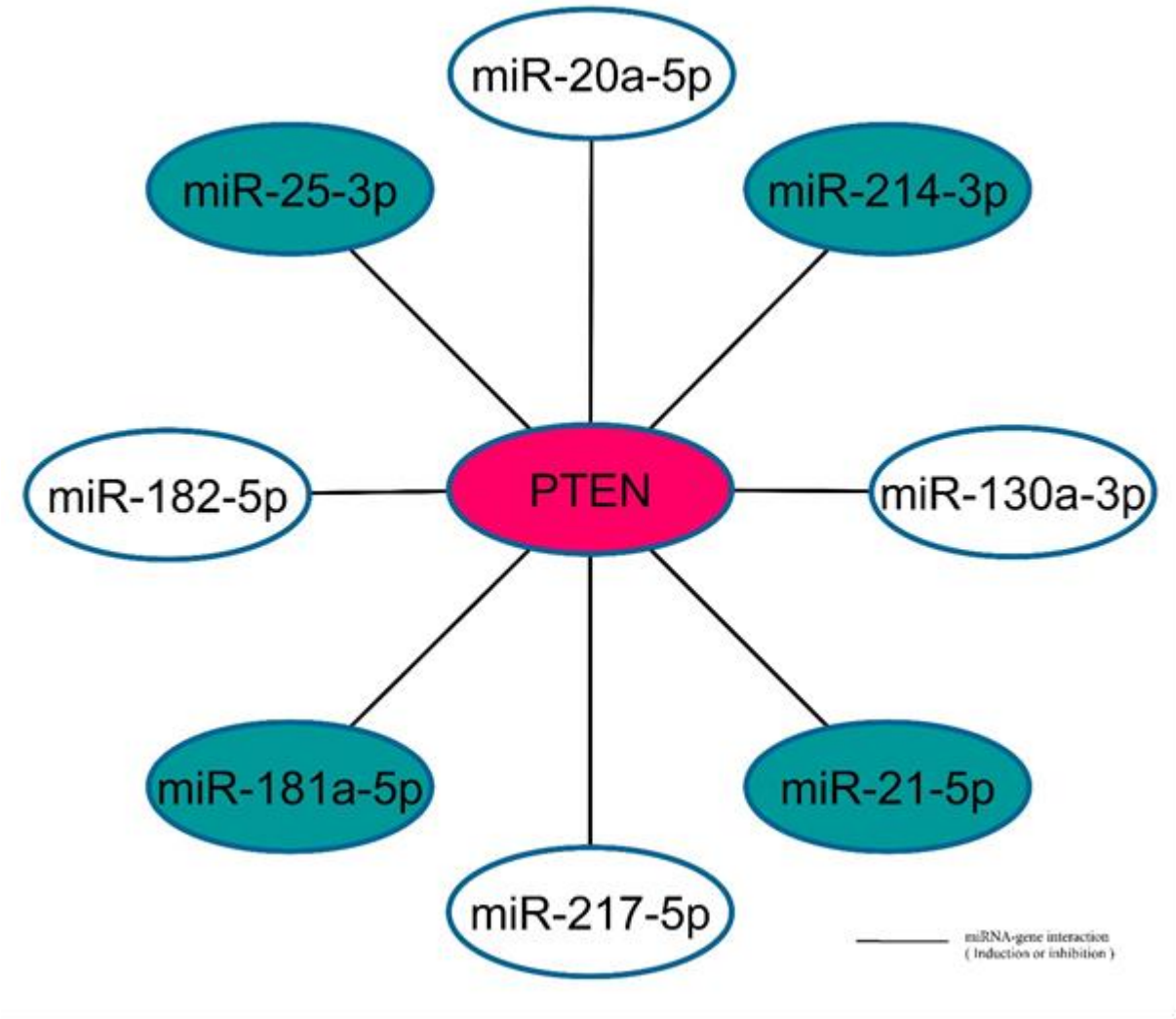

Figure 2: Interaction of PTEN as the most effective gene during chemoresistance in CC based on in silico predictions.

In Table 1,the genes which have been reported to be involved during the resistance to chemotherapy were listed[12, 75-96].All genes were inputted into miRWalk 2.0[97, 98] to find their validated interactions with miRNAs. Then, miRNA-gene interaction file which was obtained from miRWalk 2.0 inputted into galaxy project online tool[99]in order to prioritizemiRNA-gene association considering the number of interactions. Finally, two miRNAs, miR-335-5p, and miR-16-5p, with twenty-one and twenty target genes respectively,

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 April 2020 doi:10.20944/preprints202004.0294.v1

are predicted as the potential biomarkers during the resistance to chemotherapy, based on the number of the miRNA-gene interactions (Figure3).

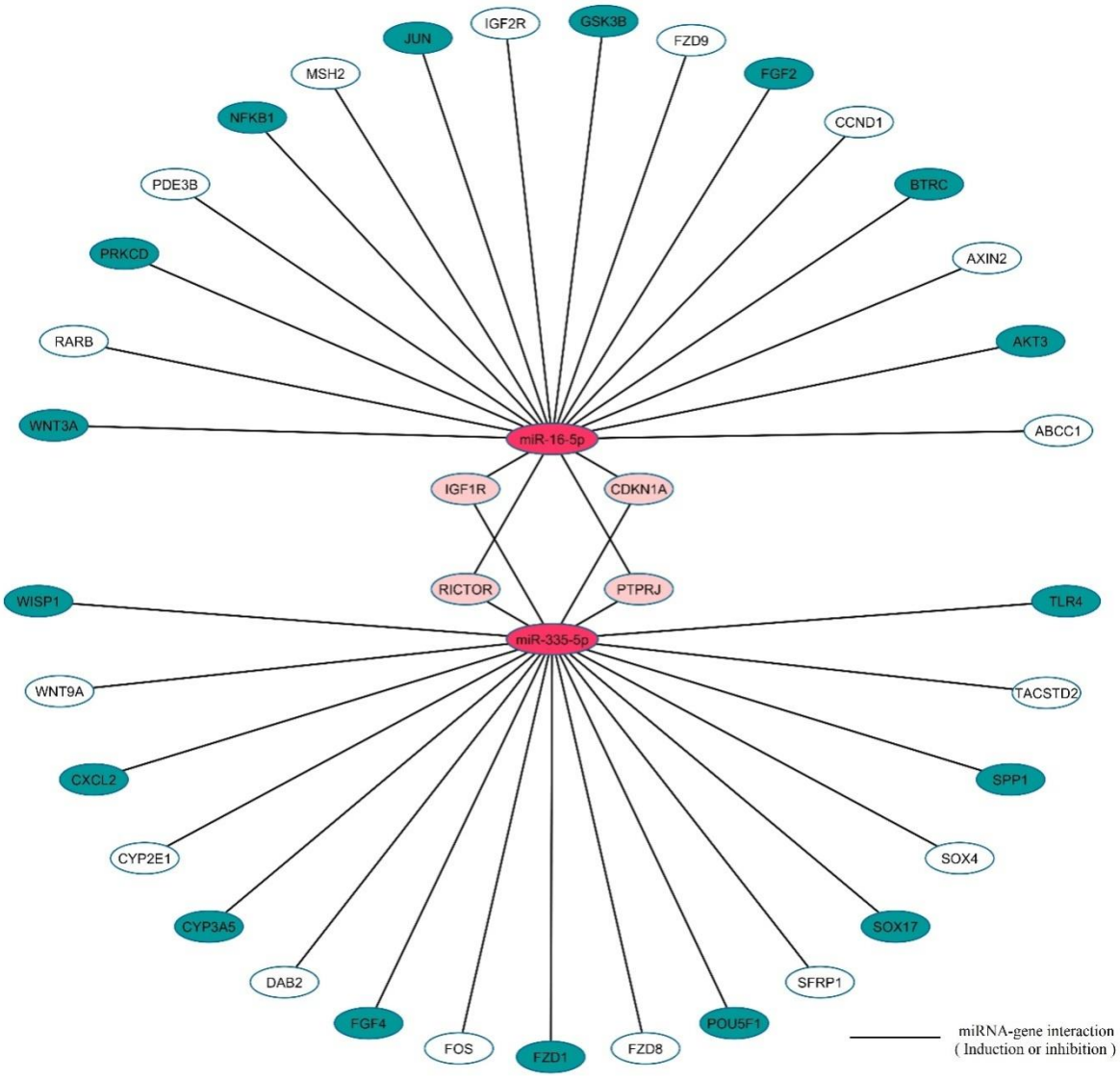

Figure 3. miR-16-5p and miR-335-5p are predicted as the two most potential biomarkers based on the number of miRNA-gene interactions for monitoring poor response to chemotherapy in CC. RICTOR, PTPRJ, IGF1R, CDKN1A, are common targets between the two mentioned miRNAs.

In the ovarian cancer cells, which are resistant to the cisplatin, miR-335-5p was downregulated. R Liu *et al*. found that the upregulation of miR-335-5p promotes cell apoptosis and induces cisplatin sensitivity by downregulating the *BCL2L2* gene in the ovarian

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 April 2020 doi:10.20944/preprints202004.0294.v1

cancer cells [100]. In another study, it was understood that miR-335 is downregulated in the CC tissues in comparison with control samples. Moreover, by transfecting the miR-33p mimic to the HeLa cells, it was proved the upregulation of miR-335 inhibits cell proliferation and invasion in the HeLa CC cells [101].

The downregulation of miR-16-5p in breast cancer has been reported. Y Qu1*etal*. showed that overexpression of miR-16-5p suppresses the proliferation and colony formation and also induces apoptosis in the breast cancer cells[13]. In CC, the expression level of miR-16 shows different expression level from low in the cervical intraepithelial neoplasia(CIN) I to high expression in CIN II-III[102].However, it has not been reported any studies about the involvement ofmiR-335-5p andmiR-16-5p in the chemoresistance in CC. Therefore, more studies are needed to find the role of miR-16-5p and miR-335-5p in the chemoresistance in CC.

CC stem cells (CSCs) and drug resistance

Based on the apparent similarities between stem cells and cancer stem cells, many researchers believe that CSCs are derived from stem cells that have mutated. CSCs are considered as a major cause of chemo and radio-resistance and also relapse after primary successful treatment. The drug resistance property of CSCs can be the result of multidrug resistance, anti-apoptotic mechanism, and increased DNA repairability, etc.[103]. Some biomarkers for CSCs drug resistance have been reported. For example, overexpression of dehydrogenase (ALDH), which considered as a biomarker for CSCs has been determined to enhance cisplatin resistance in CC cells. Moreover, W Wang *et al*. showed that the upregulation of miR- 23b can suppress the *ALDH* and promotes sensitivity of Hela and Caski cells to cisplatin[12, 104].

Long non-coding RNAs

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 April 2020 doi:10.20944/preprints202004.0294.v1

As the miRNAs, long non-coding RNAs (LncRNAs) play crucial roles in regulating biological processes such as cell cycle, growth, gene expression, and effecting the cellular cascades. In CC, there are some LncRNAs which their dysregulated expression is considered as a biomarker for chemoresistance. M BD *et al*. showed the overexpression of LINC00511 increases resistance to paclitaxel in Hela cells. Furthermore, the silencing of LINC00511 decreased the expression of *MRP1*, *P-GP*, *Bcl-2*, *MMP-2*, and *MMP-9*, while the expression level of cleaved-caspase-3 and *Bax* was increased. Altogether, it was determined that knockdown of LINC00511 could decrease cell proliferation, migration, and resistance to paclitaxel in Hela cells[105].It has been demonstrated that mutation in MutS protein homolog 2 (MSH2) could suspect women to CC. Wei-Yi Zhang *et al*. found that long noncoding RNA NCK1-AS1 bound to miR-134-5p and indirectly regulated MSH2. It was shown that knockdown of NCK1-AS1 decreased the MSH2 activity and therefore induced apoptosis in Hela cells[59].Wenjie Hou *et al*. have reported the upregulation of HOXD-AS1 in cisplatin-resistant CC cells. The knockdown of HOXD-AS1 significantly suppresses invasion and migration of cisplatin-resistant CC cells [61]. Reported that a combination of Paclitaxel with lncRNARP11-381N20.2 increases apoptosis and inhibits proliferation in CC cells.Further, the expression level of lncRNARP11-381N20.2 was significantly lower in chemo- resistant CC patients in comparison with sensitive ones[106].Cancer Susceptibility Candidate 2 (CASC2), as a long non-coding RNA has been determined to be down-regulated in cisplatin-resistant CC cells. It was found that Cancer CASC2 binds to miR-21 and inhibits it and upregulates PTEN to induce chemosensitivity of CC cells to cisplatin. In cisplatin-resistant CC cells, the expression level of CASC2 was significantly decreased [107]. Finally, LncRNA PVT1 has been introduced as an oncogenic lncRNA involved in chemoresistance. Ching-Ju Shen *et al*. proved PVT1 directly binds to miR-195, a tumor suppressor miRNA and decreases its expression. Knockdown of PVT1 restored the miR-195 expression and increased the

Paclitaxel-induced apoptosis by induction of caspase-3 and therefore sensitized the CC Caski cells to paclitaxel [108].

Conclusion

Regarding the first-line defense of chemo drugs in order to deal with the progression of several types of cancers, the incidence of chemoresistance is the main concern in cancer treatment protocols [109]. Besides the inherent chemoresistance form, the acquired types are demonstrated to result from the overactivation of some cellular signaling pathways or dysregulation of miRNAs or lncRNAs. As mentioned before, the most important role of signaling pathways, miRNAs, and lncRNAs is the overexpression of transcription factors. By using bioinformatics tools, it is also predicted that PTEN is the most potentiate proteins targeted by CC-involved miRNAs and has the major roles in CC chemoresistance. Additionally, miR-335-5p and miR-16-5p are shown to have more interactions with genes involved in CC chemoresistance. The exact functions of these miRNAs, as well as cellular signaling pathways, are needed to be investigated by further studies.

References

1. Organization, W. H. Cervical cancer. http://www.who.int/cancer/prevention/diagnosis-screening/cervical-cancer/en/
2. Roszak, A.; Lianeri, M.; Sowińska, A.; Jagodziński, P. P., CYP1A1 Ile462Val polymorphism as a risk factor in cervical cancer development in the Polish population. *Molecular diagnosis & therapy* **2014,** 18, (4), 445-450.
3. Lutkowska, A.; Roszak, A.; Jagodziński, P. P., 17β-hydroxysteroid dehydrogenase type Gene 1937 A> G Polymorphism as a Risk Factor for Cervical Cancer Progression in the Polish Population. *Pathology & Oncology Research* **2017,** 23, (2), 317-322.
4. Liu, Z.-C.; Liu, W.-D.; Liu, Y.-H.; Ye, X.-H.; Chen, S.-D., Multiple sexual partners as a potential independent risk factor for cervical cancer: a meta-analysis of epidemiological studies. *Asian Pac J Cancer Prev* **2015,** 16, (9), 3893-900.
5. Capo-Chichi, C. D.; Aguida, B.; Chabi, N. W.; Cai, Q. K.; Offrin, G.; Agossou, V. K.; Sanni, A.; Xu, X.-X., Lamin A/C deficiency is an independent risk factor for cervical cancer. *Cellular Oncology* **2016,** 39, (1), 59-68.

6. Roura, E.; Castellsagué, X.; Pawlita, M.; Travier, N.; Waterboer, T.; Margall, N.; Bosch, F. X.; De Sanjosé, S.; Dillner, J.; Gram, I. T., Smoking as a major risk factor for cervical cancer and pre-cancer: Results from the EPIC cohort. *International journal of cancer* **2014,** 135, (2), 453-466.
7. Schiffman, M.; Castle, P. E.; Jeronimo, J.; Rodriguez, A. C.; Wacholder, S., Human papillomavirus and cervical cancer. *The Lancet* **2007,** 370, (9590), 890-907.
8. Forman, D.; de Martel, C.; Lacey, C. J.; Soerjomataram, I.; Lortet-Tieulent, J.; Bruni, L.; Vignat, J.; Ferlay, J.; Bray, F.; Plummer, M., Global burden of human papillomavirus and related diseases. *Vaccine* **2012,** 30, F12-F23.
9. Mittal, S.; Banks, L., Molecular mechanisms underlying human papillomavirus E6 and E7 oncoprotein-induced cell transformation. *Mutation Research/Reviews in Mutation Research* **2017,** 772, 23-35.
10. Hemmat, N.; Baghi, H. B., Human papillomavirus E5 protein, the undercover culprit of tumorigenesis. *Infectious agents and cancer* **2018,** 13, (1), 31.
11. Eskander, R. N.; Tewari, K. S., Chemotherapy in the treatment of metastatic, persistent, and recurrent cervical cancer. *Current Opinion in Obstetrics and Gynecology* **2014,** 26, (4), 314-321.
12. Zhu, H.; Luo, H.; Zhang, W.; Shen, Z.; Hu, X.; Zhu, X., Molecular mechanisms of cisplatin resistance in cervical cancer. *Drug design, development and therapy* **2016,** 10, 1885.
13. Qu, Y.; Liu, H.; Lv, X.; Liu, Y.; Wang, X.; Zhang, M.; Zhang, X.; Li, Y.; Lou, Q.; Li, S., MicroRNA-16-5p overexpression suppresses proliferation and invasion as well as triggers apoptosis by targeting VEGFA expression in breast carcinoma. *Oncotarget* **2017,** 8, (42), 72400.
14. Raza, U.; Zhang, J. D.; Şahin, Ö., MicroRNAs: master regulators of drug resistance, stemness, and metastasis. *Journal of molecular medicine* **2014,** 92, (4), 321-336.
15. Gnoni, A.; Santini, D.; Scartozzi, M.; Russo, A.; Licchetta, A.; Palmieri, V.; Lupo, L.; Faloppi, L.; Palasciano, G.; Memeo, V., Hepatocellular carcinoma treatment over sorafenib: epigenetics, microRNAs and microenvironment. Is there a light at the end of the tunnel? *Expert opinion on therapeutic targets* **2015,** 19, (12), 1623-1635.
16. Fang, Y.; Fullwood, M. J., Roles, functions, and mechanisms of long non-coding RNAs in cancer. *Genomics, proteomics & bioinformatics* **2016,** 14, (1), 42-54.
17. Chatterjee, M.; Sengupta, S., Emerging roles of long non-coding RNAs in cancer. *Journal of biosciences* **2019,** 44, (1), 22.
18. Liu, K.; Gao, L.; Ma, X.; Huang, J.-J.; Chen, J.; Zeng, L.; Ashby, C. R.; Zou, C.; Chen, Z.-S., Long non-coding RNAs regulate drug resistance in cancer. *Molecular Cancer* **2020,** 19, (1), 1-13.
19. Xu, H.; Sun, Y.; Zeng, L.; Li, Y.; Hu, S.; He, S.; Chen, H.; Zou, Q.; Luo, B., Inhibition of cytosolic phospholipase A2 alpha increases chemosensitivity in cervical carcinoma through suppressing β-catenin signaling. *Cancer biology & therapy* **2019**, 1-10.
20. Zhang, Y.; Liu, B.; Zhao, Q.; Hou, T.; Huang, X., Nuclear localizaiton of β-catenin is associated with poor survival and chemo-/radioresistance in human cervical squamous cell cancer. *International journal of clinical and experimental pathology* **2014,** 7, (7), 3908.
21. Yang, M.; Wang, M.; Li, X.; Xie, Y.; Xia, X.; Tian, J.; Zhang, K.; Tang, A., Wnt signaling in cervical cancer? *Journal of Cancer* **2018,** 9, (7), 1277.
22. Islam, S.; Mokhtari, R.; Noman, A.; Uddin, M.; Rahman, M.; Azadi, M.; Zlotta, A.; van der Kwast, T.; Yeger, H.; Farhat, W., Sonic hedgehog (Shh) signaling promotes tumorigenicity and stemness via activation of epithelial-to-mesenchymal transition (EMT) in bladder cancer. *Molecular carcinogenesis* **2016,** 55, (5), 537-551.
23. Sharma, A.; De, R.; Javed, S.; Srinivasan, R.; Pal, A.; Bhattacharyya, S., Sonic hedgehog pathway activation regulates cervical cancer stem cell characteristics during epithelial to mesenchymal transition. *Journal of cellular physiology* **2019**.
24. Godwin, P.; Baird, A.-M.; Heavey, S.; Barr, M.; O'Byrne, K.; Gately, K. A., Targeting nuclear factor-kappa B to overcome resistance to chemotherapy. *Frontiers in oncology* **2013,** 3, 120.

25. Maruyama, W.; Shirakawa, K.; Matsui, H.; Matsumoto, T.; Yamazaki, H.; Sarca, A. D.; Kazuma, Y.; Kobayashi, M.; Shindo, K.; Takaori-Kondo, A., Classical NF-κB pathway is responsible for APOBEC3B expression in cancer cells. *Biochemical and biophysical research communications* **2016,** 478, (3), 1466-1471.
26. Liu, Y.; Li, J., Correlation between the activation of PI3K/Akt/mTOR signaling pathway and the clinical prognosis in patients with cervical cancer. *Int J Clin Exp Med* **2017,** 10, (12), 16603-16610.
27. Zhang, X.-Y.; Zhang, H.-Y.; Zhang, P.-N.; Lu, X.; Sun, H., Elevated phosphatidylinositol 3-kinase activation and its clinicopathological significance in cervical cancer. *European Journal of Obstetrics & Gynecology and Reproductive Biology* **2008,** 139, (2), 237-244.
28. Shu, X.-R.; Wu, J.; Sun, H.; Chi, L.-Q.; Wang, J.-H., PAK4 confers the malignance of cervical cancers and contributes to the cisplatin-resistance in cervical cancer cells via PI3K/AKT pathway. *Diagnostic pathology* **2015,** 10, (1), 177.
29. Aithal, M. G.; Rajeswari, N., Role of Notch signalling pathway in cancer and its association with DNA methylation. *Journal of genetics* **2013,** 92, (3), 667-675.
30. Sun, L.; Song, Y.; Tong, T.; Wu, L.; Zhang, W.; Zhan, Q., Down-modulation of Notch1 expression in cervical cancer is associated with HPV-induced carcinogenesis. *Clinical Oncology and Cancer Research* **2009,** 6, (6), 401-405.
31. Wang, L.; Guo Dai, J. Y.; Wu, W.; Zhang, W., Cervical Cancer Cell Growth, Drug Resistance, and Epithelial-Mesenchymal Transition Are Suppressed by γ-Secretase Inhibitor RO4929097. *Medical science monitor: international medical journal of experimental and clinical research* **2018,** 24, 4046.
32. Fu, Z.; Tindall, D., FOXOs, cancer and regulation of apoptosis. *Oncogene* **2008,** 27, (16), 2312.
33. Zhang, B.; Gui, L.; Zhao, X.; Zhu, L.; Li, Q., FOXO1 is a tumor suppressor in cervical cancer. *Genet Mol Res* **2015,** 14, (2), 6605-6616.
34. Zhao, C.; Lu, E.; Hu, X.; Cheng, H.; Zhang, J.-A.; Zhu, X., S100A9 regulates cisplatin chemosensitivity of squamous cervical cancer cells and related mechanism. *Cancer management and research* **2018,** 10, 3753.
35. Sánchez-Hernández, P. E.; Ramirez-Dueñas, M. G.; Albarran-Somoza, B.; García-Iglesias, T.; del Toro-Arreola, A.; Franco-Topete, R.; Daneri-Navarro, A., Protease-activated receptor-2 (PAR-2) in cervical cancer proliferation. *Gynecologic oncology* **2008,** 108, (1), 19-26.
36. de Almeida, V. H.; dos Santos Guimarães, I.; Almendra, L. R.; Rondon, A. M.; Tilli, T. M.; de Melo, A. C.; Sternberg, C.; Monteiro, R. Q., Positive crosstalk between EGFR and the TF-PAR2 pathway mediates resistance to cisplatin and poor survival in cervical cancer. *Oncotarget* **2018,** 9, (55), 30594.
37. Soonthornthum, T.; Arias-Pulido, H.; Joste, N.; Lomo, L.; Muller, C.; Rutledge, T.; Verschraegen, C., Epidermal growth factor receptor as a biomarker for cervical cancer. *Annals of oncology* **2011,** 22, (10), 2166-2178.
38. Hemmat, N.; Baghi, H. B., Human papillomavirus E5 protein, the undercover culprit of tumorigenesis. *Infect Agent Cancer* **2018,** 13, (1), 31.
39. Rawlings, J. S.; Rosler, K. M.; Harrison, D. A., The JAK/STAT signaling pathway. *Journal of cell science* **2004,** 117, (8), 1281-1283.
40. Huang, L.; Rao, W., SiRNA interfering STAT3 enhances DDP sensitivity in cervical cancer cells. *European review for medical and pharmacological sciences* **2018,** 22, 4098-4106.
41. Gurung, A. B.; Bhattacharjee, A., Significance of Ras Signaling in Cancer and Strategies for its Control. *Journal-Significance of Ras Signaling in Cancer and Strategies for its Control* **2015**.
42. Yoshida, S.; Kajitani, N.; Satsuka, A.; Nakamura, H.; Sakai, H., Ras modifies proliferation and invasiveness of cells expressing human papillomavirus oncoproteins. *Journal of virology* **2008,** 82, (17), 8820-8827.

43. Mammas, I. N.; Zafiropoulos, A.; Koumantakis, E.; Sifakis, S.; Spandidos, D. A., Transcriptional activation of H-and N-ras oncogenes in human cervical cancer. *Gynecologic oncology* **2004,** 92, (3), 941-948.
44. Pan, Q.; Liu, R.; Banu, H.; Ma, L.; Li, H., Inhibition of isoprenylcysteine carboxylmethyltransferase sensitizes common chemotherapies in cervical cancer via Ras-dependent pathway. *Biomedicine & Pharmacotherapy* **2018,** 99, 169-175.
45. Xu, J.; Pan, Q.; Ju, W., Ras inhibition by zoledronic acid effectively sensitizes cervical cancer to chemotherapy. *Anti-cancer drugs* **2019**.
46. He, C.; Mao, D.; Hua, G.; Lv, X.; Chen, X.; Angeletti, P. C.; Dong, J.; Remmenga, S. W.; Rodabaugh, K. J.; Zhou, J., The Hippo/YAP pathway interacts with EGFR signaling and HPV oncoproteins to regulate cervical cancer progression. *EMBO molecular medicine* **2015,** 7, (11), 1426-1449.
47. Bi, L.; Ma, F.; Tian, R.; Zhou, Y.; Lan, W.; Song, Q.; Cheng, X., AJUBA increases the cisplatin resistance through hippo pathway in cervical cancer. *Gene* **2018,** 644, 148-154.
48. Tao, Y.; Hu, K.; Tan, F.; Zhang, S.; Zhou, M.; Luo, J.; Wang, Z., SH3-domain binding protein 1 in the tumor microenvironment promotes hepatocellular carcinoma metastasis through WAVE2 pathway. *Oncotarget* **2016,** 7, (14), 18356.
49. Wang, J.; Feng, Y.; Chen, X.; Du, Z.; Jiang, S.; Ma, S.; Zou, W., SH3BP1-induced Rac-Wave2 pathway activation regulates cervical cancer cell migration, invasion, and chemoresistance to cisplatin. *Journal of cellular biochemistry* **2018,** 119, (2), 1733-1745.
50. Vicentini, C.; Fassan, M.; D'Angelo, E.; Corbo, V.; Silvestris, N.; Nuovo, G. J.; Scarpa, A., Clinical application of microRNA testing in neuroendocrine tumors of the gastrointestinal tract. *Molecules* **2014,** 19, (2), 2458-2468.
51. An, X.; Sarmiento, C.; Tan, T.; Zhu, H., Regulation of multidrug resistance by microRNAs in anti-cancer therapy. *Acta pharmaceutica sinica B* **2017,** 7, (1), 38-51.
52. Rukov, J. L.; Wilentzik, R.; Jaffe, I.; Vinther, J.; Shomron, N., Pharmaco-miR: linking microRNAs and drug effects. *Briefings in bioinformatics* **2013,** 15, (4), 648-659.
53. Si, W.; Shen, J.; Zheng, H.; Fan, W., The role and mechanisms of action of microRNAs in cancer drug resistance. *Clinical epigenetics* **2019,** 11, (1), 25.
54. Yin, Z.; Ren, W., Microrna-217 acts as a tumor suppressor and correlates with the chemoresistance of cervical carcinoma to cisplatin. *OncoTargets and therapy* **2019,** 12, 759.
55. Tyagi, A.; Vishnoi, K.; Kaur, H.; Srivastava, Y.; Roy, B. G.; Das, B. C.; Bharti, A. C., Cervical cancer stem cells manifest radioresistance: Association with upregulated AP-1 activity. *Scientific reports* **2017,** 7, (1), 4781.
56. Yao, T.; Lu, R.; Zhang, J.; Fang, X.; Fan, L.; Huang, C.; Lin, R.; Lin, Z., Growth arrest-specific 5 attenuates cisplatin-induced apoptosis in cervical cancer by regulating STAT3 signaling via miR-21. *Journal of Cellular Physiology* **2019,** 234, (6), 9605-9615.
57. Wen, Q.; Liu, Y.; Lyu, H.; Xu, X.; Wu, Q.; Liu, N.; Yin, Q.; Li, J.; Sheng, X., Long noncoding RNA GAS5, which acts as a tumor suppressor via microRNA 21, regulates cisplatin resistance expression in cervical cancer. *International Journal of Gynecologic Cancer* **2017,** 27, (6), 1096-1108.
58. Zhang, J.; Li, Y.-H.; Liu, H.-L.; Zhang, Y.; Zhang, Q.-S.; Li, S.-Z., Correlations of MicroRNA-21 Gene Polymorphisms With Chemosensitivity and Prognosis of Cervical Cancer. *The American journal of the medical sciences* **2018,** 356, (6), 544-551.
59. Zhang, W. Y.; Liu, Y. J.; He, Y.; Chen, P., Suppression of long noncoding RNA NCK1-AS1 increases chemosensitivity to cisplatin in cervical cancer. *Journal of cellular physiology* **2019,** 234, (4), 4302-4313.
60. Feng, C.; Ma, F.; Hu, C.; Ma, J.-a.; Wang, J.; Zhang, Y.; Wu, F.; Hou, T.; Jiang, S.; Wang, Y., SOX9/miR-130a/CTR1 axis modulates DDP-resistance of cervical cancer cell. *Cell Cycle* **2018,** 17, (4), 448-458.

61. Chi, C.; Mao, M.; Shen, Z.; Chen, Y.; Chen, J.; Hou, W., HOXD-AS1 exerts oncogenic functions and promotes chemoresistance in cisplatin-resistant cervical cancer cells. *Human gene therapy* **2018,** 29, (12), 1438-1448.
62. Wang, F.; Liu, M.; Li, X.; Tang, H., MiR-214 reduces cell survival and enhances cisplatin-induced cytotoxicity via down-regulation of Bcl2l2 in cervical cancer cells. *FEBS letters* **2013,** 587, (5), 488-495.
63. Wen, Z.; Lei, Z.; Jin-An, M.; Xue-Zhen, L.; Xing-Nan, Z.; Xiu-Wen, D., The inhibitory role of miR-214 in cervical cancer cells through directly targeting mitochondrial transcription factor A (TFAM). *Eur J Gynaecol Oncol* **2014,** 35, (6), 676-682.
64. Zhu, H.; Wu, H.; Liu, X.; Evans, B. R.; Medina, D. J.; Liu, C.-G.; Yang, J.-M., Role of MicroRNA miR-27a and miR-451 in the regulation of MDR1/P-glycoprotein expression in human cancer cells. *Biochemical pharmacology* **2008,** 76, (5), 582-588.
65. Danza, K.; Silvestris, N.; Simone, G.; Signorile, M.; Saragoni, L.; Brunetti, O.; Monti, M.; Mazzotta, A.; De Summa, S.; Mangia, A., Role of miR-27a, miR-181a and miR-20b in gastric cancer hypoxia-induced chemoresistance. *Cancer biology & therapy* **2016,** 17, (4), 400-406.
66. Xiong, Y.; Sun, F.; Dong, P.; Watari, H.; Yue, J.; Yu, M.-f.; Lan, C.-y.; Wang, Y.; Ma, Z.-b., iASPP induces EMT and cisplatin resistance in human cervical cancer through miR-20a-FBXL5/BTG3 signaling. *Journal of Experimental & Clinical Cancer Research* **2017,** 36, (1), 48.
67. Phuah, N. H.; Azmi, M. N.; Awang, K.; Nagoor, N. H., Suppression of microRNA-629 enhances sensitivity of cervical cancer cells to 1′ S-1′-acetoxychavicol acetate via regulating RSU1. *OncoTargets and therapy* **2017,** 10, 1695.
68. Song, J.; Li, Y., miR-25-3p reverses epithelial-mesenchymal transition via targeting Sema4C in cisplatin-resistance cervical cancer cells. *Cancer science* **2017,** 108, (1), 23-31.
69. Yang, Y.; Dai, C.; Cai, Z.; Hou, A.; Cheng, D.; Wu, G.; Li, J.; Cui, J.; Xu, D., The pathway analysis of micrornas regulated drug-resistant responses in HeLa cells. *IEEE transactions on nanobioscience* **2016,** 15, (2), 113-118.
70. Lee, J. H.; Kim, C.; Sethi, G.; Ahn, K. S., Brassinin inhibits STAT3 signaling pathway through modulation of PIAS-3 and SOCS-3 expression and sensitizes human lung cancer xenograft in nude mice to paclitaxel. *Oncotarget* **2015,** 6, (8), 6386.
71. Fan, Z.; Cui, H.; Yu, H.; Ji, Q.; Kang, L.; Han, B.; Wang, J.; Dong, Q.; Li, Y.; Yan, Z., MiR-125a promotes paclitaxel sensitivity in cervical cancer through altering STAT3 expression. *Oncogenesis* **2016,** 5, (2), e197.
72. Chen, Y.; Ke, G.; Han, D.; Liang, S.; Yang, G.; Wu, X., MicroRNA-181a enhances the chemoresistance of human cervical squamous cell carcinoma to cisplatin by targeting PRKCD. *Experimental cell research* **2014,** 320, (1), 12-20.
73. Luo, C.; Qiu, J., MiR-181a inhibits cervical cancer development via downregulating GRP78. *Oncology Research Featuring Preclinical and Clinical Cancer Therapeutics* **2017,** 25, (8), 1341-1348.
74. Leslie, N. R.; Downes, C. P., PTEN function: how normal cells control it and tumour cells lose it. *Biochemical Journal* **2004,** 382, (1), 1-11.
75. Brozovic, A.; Fritz, G.; Christmann, M.; Zisowsky, J.; Jaehde, U.; Osmak, M.; Kaina, B., Long-term activation of SAPK/JNK, p38 kinase and fas-L expression by cisplatin is attenuated in human carcinoma cells that acquired drug resistance. *International journal of cancer* **2004,** 112, (6), 974-985.
76. Yeh, P. Y.; Chuang, S.-E.; Yeh, K.-H.; Song, Y. C.; Cheng, A.-L., Involvement of nuclear transcription factor-κB in low-dose doxorubicin-induced drug resistance of cervical carcinoma cells. *Biochemical pharmacology* **2003,** 66, (1), 25-33.
77. De Schutter, T.; Andrei, G.; Topalis, D.; Duraffour, S.; Mitera, T.; van den Oord, J.; Matthys, P.; Snoeck, R., Reduced tumorigenicity and pathogenicity of cervical carcinoma SiHa cells selected for resistance to cidofovir. *Molecular cancer* **2013,** 12, (1), 158.

78. Chen, X.; Xiong, D.; Ye, L.; Yang, H.; Mei, S.; Wu, J.; Chen, S.; Mi, R., SPP1 inhibition improves the cisplatin chemo-sensitivity of cervical cancer cell lines. *Cancer chemotherapy and pharmacology* **2019,** 83, (4), 603-613.
79. Sun, R.; Jiang, B.; Qi, H.; Zhang, X.; Yang, J.; Duan, J.; Li, Y.; Li, G., SOX4 contributes to the progression of cervical cancer and the resistance to the chemotherapeutic drug through ABCG2. *Cell death & disease* **2015,** 6, (11), e1990.
80. Deng, H. B.; Adikari, M.; Parekh, H. K.; Simpkins, H., Ubiquitous induction of resistance to platinum drugs in human ovarian, cervical, germ-cell and lung carcinoma tumor cells overexpressing isoforms 1 and 2 of dihydrodiol dehydrogenase. *Cancer chemotherapy and pharmacology* **2004,** 54, (4), 301-307.
81. Maeda, M.; Watanabe, N.; Okamoto, T.; Yoshiro Niitsu, N. T., Endogenous tumor necrosis factor functions as a resistant factor against adriamycin. *International journal of cancer* **1994,** 58, (3), 376-379.
82. Takahashi, N.; Yanagihara, M.; Ogawa, Y.; Yamanoha, B.; Andoh, T., Down-regulation of Bcl-2-interacting protein BAG-1 confers resistance to anti-cancer drugs. *Biochemical and biophysical research communications* **2003,** 301, (3), 798-803.
83. Oh, J.-M.; Ryoo, I.-J.; Yang, Y.; Kim, H.-S.; Yang, K.-H.; Moon, E.-Y., Hypoxia-inducible transcription factor (HIF)-1α stabilization by actin-sequestering protein, thymosin beta-4 (TB4) in Hela cervical tumor cells. *Cancer letters* **2008,** 264, (1), 29-35.
84. Li, J.; Ping, Z.; Ning, H., MiR-218 impairs tumor growth and increases chemo-sensitivity to cisplatin in cervical cancer. *International journal of molecular sciences* **2012,** 13, (12), 16053-16064.
85. Uzawa, K.; Kasamatsu, A.; Baba, T.; Usukura, K.; Saito, Y.; Sakuma, K.; Iyoda, M.; Sakamoto, Y.; Ogawara, K.; Shiiba, M., Targeting phosphodiesterase 3 B enhances cisplatin sensitivity in human cancer cells. *Cancer medicine* **2013,** 2, (1), 40-49.
86. Park, J.-H.; Yoon, D.-S.; Choi, H.-J.; Hahm, D.-H.; Oh, S.-M., Phosphorylation of IκBα at serine 32 by T-lymphokine-activated killer cell-originated protein kinase is essential for chemoresistance against doxorubicin in cervical cancer cells. *Journal of Biological Chemistry* **2013,** 288, (5), 3585-3593.
87. Wanichwatanadecha, P.; Sirisrimangkorn, S.; Kaewprag, J.; Ponglikitmongkol, M., Transactivation activity of human papillomavirus type 16 E6* I on aldo-keto reductase genes enhances chemoresistance in cervical cancer cells. *Journal of general virology* **2012,** 93, (5), 1081-1092.
88. Okada, T.; Murata, K.; Hirose, R.; Matsuda, C.; Komatsu, T.; Ikekita, M.; Nakawatari, M.; Nakayama, F.; Wakatsuki, M.; Ohno, T., Upregulated expression of FGF13/FHF2 mediates resistance to platinum drugs in cervical cancer cells. *Scientific reports* **2013,** 3, 2899.
89. Yim, E.-K.; Tong, S.-Y.; Ho, E.-M.; Bae, J.-H.; Um, S.-J.; Park, J.-S., Anticancer effects on TACC3 by treatment of paclitaxel in HPV-18 positive cervical carcinoma cells. *Oncology reports* **2009,** 21, (2), 549-557.
90. Yan, C.-M.; Zhao, Y.-L.; Cai, H.-Y.; Miao, G.-Y.; Ma, W., Blockage of PTPRJ promotes cell growth and resistance to 5-FU through activation of JAK1/STAT3 in the cervical carcinoma cell line C33A. *Oncology reports* **2015,** 33, (4), 1737-1744.
91. Yang, F.; Gao, B.; Li, R.; Li, W.; Chen, W.; Yu, Z.; Zhang, J., Expression levels of resistant genes affect cervical cancer prognosis. *Molecular medicine reports* **2017,** 15, (5), 2802-2806.
92. Liu, X.; Wang, D.; Liu, H.; Feng, Y.; Zhu, T.; Zhang, L.; Zhu, B.; Zhang, Y., Knockdown of astrocyte elevated gene-1 (AEG-1) in cervical cancer cells decreases their invasiveness, epithelial to mesenchymal transition, and chemoresistance. *Cell Cycle* **2014,** 13, (11), 1702-1707.
93. Wang, F.; Liu, X.; Yang, P.; Guo, L.; Liu, C.; Li, H.; Long, S.; Shen, Y.; Wan, H., Loss of TACSTD2 contributed to squamous cell carcinoma progression through attenuating TAp63-dependent apoptosis. *Cell death & disease* **2014,** 5, (3), e1133.

94. Britten, R. A.; Liu, D.; Tessier, A.; Hutchison, M. J.; Murray, D., ERCC1 expression as a molecular marker of cisplatin resistance in human cervical tumor cells. *International journal of cancer* **2000,** 89, (5), 453-457.
95. Mutlu, P.; Azarkan, S. Y.; Pourianazar, N. T.; Yücel, M.; Gündüz, U., Determination of the relationship between doxorubicin resistance and Wnt signaling pathway in HeLa and K562 cell lines. *EXCLI journal* **2018,** 17, 386.
96. Li, Q.; Peng, X.; Yang, H.; Rodriguez, J.-A.; Shu, Y., Contribution of organic cation transporter 3 to cisplatin cytotoxicity in human cervical cancer cells. *Journal of pharmaceutical sciences* **2012,** 101, (1), 394-404.
97. Dweep, H.; Sticht, C.; Pandey, P.; Gretz, N., miRWalk–database: prediction of possible miRNA binding sites by "walking" the genes of three genomes. *Journal of biomedical informatics* **2011,** 44, (5), 839-847.
98. Dweep, H.; Gretz, N., miRWalk2. 0: a comprehensive atlas of microRNA-target interactions. *Nature methods* **2015,** 12, (8), 697.
99. Afgan, E.; Baker, D.; Van den Beek, M.; Blankenberg, D.; Bouvier, D.; Čech, M.; Chilton, J.; Clements, D.; Coraor, N.; Eberhard, C., The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2016 update. *Nucleic acids research* **2016,** 44, (W1), W3-W10.
100. Liu, R.; Guo, H.; Lu, S., MiR-335-5p restores cisplatin sensitivity in ovarian cancer cells through targeting BCL2L2. *Cancer medicine* **2018,** 7, (9), 4598-4609.
101. Chen, L.; Song, Y.; Lu, Y.; Zheng, W.; Ma, W.; Zhang, C., miR-335 inhibits cell proliferation, migration and invasion in HeLa cervical cancer cells. *Int J Clin Exp Pathol* **2016,** 9, (10), 10351-10362.
102. Sharma, G.; Dua, P.; Mohan Agarwal, S., A comprehensive review of dysregulated miRNAs involved in cervical cancer. *Current genomics* **2014,** 15, (4), 310-323.
103. Yao, T.; Lu, R.; Zhang, Y.; Zhang, Y.; Zhao, C.; Lin, R.; Lin, Z., Cervical cancer stem cells. *Cell proliferation* **2015,** 48, (6), 611-625.
104. Wang, W.; Li, Y.; Liu, N.; Gao, Y.; Li, L., MiR-23b controls ALDH1A1 expression in cervical cancer stem cells. *BMC cancer* **2017,** 17, (1), 292.
105. Mao, B.-D.; Xu, P.; Zhong, Y.; Ding, W.-W.; Meng, Q.-Z., LINC00511 knockdown prevents cervical cancer cell proliferation and reduces resistance to paclitaxel. *Journal of biosciences* **2019,** 44, (2), 44.
106. Zou, S.; Du, X.; Lin, H.; Wang, P.; Li, M., Paclitaxel inhibits the progression of cervical cancer by inhibiting autophagy via lncRNARP11-381N20. 2. *Eur Rev Med Pharmacol Sci* **2018,** 22, (10), 3010-3017.
107. Feng, Y.; Zou, W.; Hu, C.; Li, G.; Zhou, S.; He, Y.; Ma, F.; Deng, C.; Sun, L., Modulation of CASC2/miR-21/PTEN pathway sensitizes cervical cancer to cisplatin. *Archives of biochemistry and biophysics* **2017,** 623, 20-30.
108. Shen, C.-J.; Cheng, Y.-M.; Wang, C.-L., LncRNA PVT1 epigenetically silences miR-195 and modulates EMT and chemoresistance in cervical cancer cells. *Journal of drug targeting* **2017,** 25, (7), 637-644.
109. Jo, Y.; Choi, N.; Kim, K.; Koo, H. J.; Choi, J.; Kim, H. N., Chemoresistance of Cancer Cells: Requirements of Tumor Microenvironment-mimicking In Vitro Models in Anti-Cancer Drug Development. *Theranostics* **2018,** 8, (19), 5259-5275.

 doi:10.20944/preprints202004.0294.v1

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 April 2020 doi:10.20944/preprints202004.0294.v1

Genes	Dysregulation trend	Drug Resistance	Experimental condition	Genes	Dysregulation trend	Drug resistance	Experimental condition
AXIN2	Up	Doxorubicin	HeLa cells	APC	Up	Doxorubicin	K652 cells
DKK1	Down	Doxorubicin	HeLa cells	AXIN2	Down	Doxorubicin	K652 cells
FRAT1	Down	Doxorubicin	HeLa/K652	CXXC4	Down	Doxorubicin	K652 cells
FZD1	Down	Doxorubicin	HeLa cells	FZD1	Up	Doxorubicin	K652 cells
FZD7	Down	Doxorubicin	HeLa cells	FZD9	Down	Doxorubicin	K652 cells
FZD8	Down	Doxorubicin	HeLa cells	SFRP1	Up	Doxorubicin	K652 cells
GSK3B	Down	Doxorubicin	HeLa cells	Wnt1	Down	Doxorubicin	K652 cells
NKD1	Down	Doxorubicin	HeLa cells	RHOU	Up	Doxorubicin	K652 cells
SOX17	Down	Doxorubicin	HeLa cells	Wnt11	Down	Doxorubicin	K652 cells
Wnt10A	Down	Doxorubicin	HeLa cells	Wnt5B	Up	Doxorubicin	K652 cells
Wnt3A	Down	Doxorubicin	HeLa cells	DAB2	Down	Doxorubicin	K652 cells
Wnt7A	Down	Doxorubicin	HeLa cells	PYGO1	Up	Doxorubicin	K652 cells
PRICKLE1	Up	Doxorubicin	HeLa cells	BAX	Down	Doxorubicin	K652 cells
RHOU	Down	Doxorubicin	HeLa cells	TOP2A	Down	Doxorubicin	K652 cells
NFATC1	Down	Doxorubicin	HeLa cells	CYP3A5	Up	Doxorubicin	K652 cells
Wnt11	Up	Doxorubicin	HeLa cells	MSH2	Up	Doxorubicin	K652 cells
BTRC	Down	Doxorubicin	HeLa cells	ERBB2	Down	Doxorubicin	K652 cells
FRZB	Down	Doxorubicin	HeLa/K652	IGF1R	Down	Doxorubicin	K652 cells
JUN	Down	Doxorubicin	HeLa cells	IGF2R	Up	Doxorubicin	K652 cells

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 April 2020 doi:10.20944/preprints202004.0294.v1

MMP7	Up	Doxorubicin	HeLa cells	MET	Down	Doxorubicin	K652 cells
WISP1	Down	Doxorubicin	HeLa cells	PPARD	Down	Doxorubicin	K652 cells
FGF4	Down	Doxorubicin	HeLa cells	RARB	Down	Doxorubicin	K652 cells
FOXN1	Down	Doxorubicin	HeLa/K652	ELK1	Up	Doxorubicin	K652 cells
CASP9	Down	Doxorubicin	HeLa cells	HIF1A	Down	Doxorubicin	K652 cells
SOCS3	Down	Doxorubicin	HeLa/K652	NFKB1	Up	Doxorubicin	K652 cells
DKK3	Up	Doxorubicin	HeLa cells	NFKBIE	Down	Doxorubicin	K652 cells
PYGO1	Down	Doxorubicin	HeLa cells	FAS	Up	Doxorubicin	K652 cells
TCF7	Down	Doxorubicin	HeLa cells	GAS5	Down	cisplatin	Tissue sample
TCFL1	Down	Doxorubicin	HeLa cells	Akt3	Up	cisplatin	HeLa cells
Wnt2B	Down	Doxorubicin	HeLa cells	SOX4	Up	cisplatin	Caski
Wnt8A	Down	Doxorubicin	HeLa cells	ABCG2	Up	cisplatin	Caski
Wnt9A	Down	Doxorubicin	HeLa/K652	PTPRJ	Down	5-fu	C33A cell
FBXW4	Down	Doxorubicin	HeLa cells	MDR1	Up	Paclitaxel/	HeLa
KREMEN1	Down	Doxorubicin	HeLa cells			cisplatin	
TLE1	Up	Doxorubicin	HeLa cells	AEG-1	Up	paclitaxel/	HeLa cells
FZD5	Down	Doxorubicin	HeLa cells			cisplatin	
ABCB1	Up	Doxorubicin	HeLa/K652	TACSTD2	Down	Unknown	squamous cell
ABCC1	Down	Doxorubicin	HeLa/K652				carcinoma
ABCC2	Down	Doxorubicin	HeLa cells				tissue
ABCG2	Up	Doxorubicin	HeLa cells	TGFB1	Down	Cidofovir	SiHa cells

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 April 2020 doi:10.20944/preprints202004.0294.v1

BAX	Up	Doxorubicin	HeLa cells	STAT3	Down	Cidofovir	SiHa cells
TOP2B	Up	Doxorubicin	HeLa cells	SOCS3	Down	Cidofovir	SiHa cells
BLMH	Down	Doxorubicin	HeLa/K652	TLR3	Down	Cidofovir	SiHa cells
CYP2E1	Down	Doxorubicin	HeLa cells	TLR4	Down	Cidofovir	SiHa cells
CCND1	Up	Doxorubicin	HeLa cells	CCND1	Up	cidofovir	SiHa cells
SOD1	Up	Doxorubicin	HeLa cells	CXCL2	Up	cidofovir	SiHa cells
CDKN1A	Up	Doxorubicin	HeLa cells	CEBPB	Up	cidofovir	SiHa cells
FGF2	Up	Doxorubicin	HeLa cells	STAT1	Up	cidofovir	SiHa cells
PPARG	Down	Doxorubicin	HeLa cells	AKT3	Down	cidofovir	SiHa cells
AP1S1	Up	Doxorubicin	HeLa/K652	MAPK	Down	cidofovir	SiHa cells
FOS	Up	Doxorubicin	HeLa cells	PRKCD	Down	Cisplatin	squamous cell
NFKB1	Down	Doxorubicin	HeLa cells				carcinoma
NFKB2	Down	Doxorubicin	HeLa cells				tissue
TNFRSF11	Down	Doxorubicin	HeLa cells	PDE3B	Up	Cisplatin	HeLa cells
TOPK	Up	Doxorubicin	HeLa cells	FGF13	Up	Cisplatin	HeLa cells
AKR1C	Up	Doxorubicin	CC3 cells	RICTOR	Up	Cisplatin	HeLa cells
TACC3	Up	Paclitaxel	Cervical cancer cells	OCT3	Down	Cisplatin	KB-CP20 cell
				TB4P	Up	paclitaxel	HeLa cells
JNK	Down	Cisplatin	HeLa cells	p38	Down	Cisplatin	HeLa cells
c-Jun	Down	Cisplatin	HeLa cells	DDH	Up	Cisplatin	A431 cells
NFKB1	Up	Doxorubicin	SiHa cells	ERCC1	Up	Cisplatin	CC cells

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 17 April 2020 doi:10.20944/preprints202004.0294.v1

TNF	**Up**	**Adriamycin**	**HeLa cells**
BAG-1	**Down**	**Multidrug**	**HeLa cells**
SPP1	**Up**	**Cisplatin**	**HeLa cells**
AKR1C1	**Up**	**multidrug**	**C33A cells**

Table 1: The genes involved in chemoresistance of Cervical Cancer