ACTA SCIENTIFIC CANCER BIOLOGY

Volume 3 Issue 11 November 2019

Case Study

A Systematic Review of Aetiopathogenesis of Cervical Cancer Development-Role of HPV, Cervical Cancer Stem Cells and Micro RNA and Utilizing these as Novel Targets for Treatment – An Update

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Received: September 03, 2019; **Published:** October 24, 2019

Abstract

One of the most important causes of deaths due to cancer are related to cervical cancer as in most developing countries. Aetiology has a lot of factors although most important ones remain human papilloma virus (HPV) infection-Specifically strain 16 and 18. Here we further emphasize on roles of its protein E1-E7, L1, L2. Further role of cancer stem cells (CSC's) has been proven beyond doubt in determining tumor size, rate of tumor growth and amount of regression after therapy, hence presence of CSC's carry a poor prognosis in umpteen types of cancer including pancreatic, colonic esophageal, liver breast, prostate, lung and gastric carcinoma. As far as cervical cancer is concerned presence of CSC's imply resistance to conventionally utilized drugs like cisplatin. Thus in this review we have summarized how HPV acts to cause cervical; cancer (CC) along with implications of various pathways be it the embryological stem cell markers like NANOG, OCT4 or mesenchymal markers like vimentin etc. and importance of epithelial mesenchymal transition (EMT) specifically in the development of CC in the transitional zone of the cervix and further in spinous layer of cervical epithelium based on viral growth. Further role of miR's in affecting ALDH1 is further examined to suppress tumor spheres cell (TCs) formation to develop novel therapeutic strategies.

Keywords: HPV; CC; CSC; TCs; miR; ALDH1

Introduction

In 2015, >8. 8 million cancer-associated morbidities were reported worldwide, of these 70% occurred in low and middle income countries and 25% of these were induced by viral infections, like hepatitis virus or human papilloma virus (HPV) [1,2]. HPV 16 and 18 are the important biological causes that are associated with cervical cancer (CC) development, and are the 3rd most common causes of malignant tumors. As per the GLOBOCAN, CC remains the 4th most common cause of cancer associated mortalities among women globally [1, 2] and one of the most fatal types of cancer in the females in developing countries [2]. In Mexico, CC is the 2nd most common cause of cancer associated mortality in women (2018), mainly secondary to poor clinical diagnosis during the early stage of the disease [2]. Earlier reports between 2015 and 2017 showed that HPV 16 and 18 are responsible for65-75% of the precancerous cervical lesions and are present in 99% cases having CC all over the world [3,5]. Although it has also been proved that HPV Infections do not necessarily trigger CC [3,5]. This way a number of factors are responsible ;like contraceptive pills, multiple sexual partners, multiple births, obesity, smoking, alcoholism, poor diet, immunosuppressive cervical microenvironment, abnormal vaginal microbiota, co-infections with Chlamydia trachomatis or human immunodeficiency virus (HIV) and the presence of cervical cancer stem cells (CCSCs) [3,5].

As far as abnormal vaginal microbiota, some reports indicated marked changes in the microenvironment, of patients in the last stages of the disease. Due to this patients positive for HPV demonstrate the presence of Lactobacillus liners; however Sneathia spp., Megasphaera elsdenii and Shuttle worthia satelles were commonly found in cervical epithelial neoplasia (CIN) cases [3,5]. Thus some of the microbes can be thought to be microbiological markers associated with the HPV –infection and cervical epithelial neoplasia (CIN). Moreover Fusobacterium spp. is the most abundant microorganisms found in CC cases [4]. The changes in cervical microbiota through the progression of CIN1 to CC shown in figure 1.

Though a lot of genetic as well as molecular events need more clarification in cervical cancer onset. What is accepted is that HPV Viruses depend on epithelial cell differentiation for establishing their progeny and spread their viral genes [5]. HPV has a more opportunity to infect basal cells with stem cell like properties and integrate its viral DNA into genome of these cells, that drive the oncogenic conversion into cervical cancer stem cells (CCSCs) [5]. The infection takes place where the ectocervix and endocervix join called the transformation zone (TZ) or the squamocolumnar junction [5,6]. The TZ has a unique morphology and genetic expression profile, expressing keratin 7, anterior gradient 2, CD63 and matrix metalloproteinase 7, as well as other genes [6]. Other important factor is the epithelial-mesenchymal transition (EMT). EMT is a key process in the formation of invasive cells and metastasis and is regulated by the snail family transcriptional repressors metastases (SNAI1) and 2 (SNAI2), and twist family bHLH transcriptional factor 1 (TWIST1) transcription factors. The EMT is a rich source of CSC's and its induction promotes metastases, tumor cell invasion and drug resistance. EMT specific changes are loss of E-cadherin and Vimentin [7].

Another factor is the presence of CCSCs which has equal value in triggering CC development, since they give various malignant properties to the tumor, like proliferation and metastasis capacity, resistance to radiation and cisplatin-based chemotherapy. At present there are no therapies that can remove CCSC's and if present, they cause a poor prognosis in CC. Thus, developing dual treatments could remove both primary cancer cells and CCSC's. Particular biomarkers from CSC's are being considered as promising target for the development of newer therapies in many types of cancer.

Thus, we decided to review the important factors that are instrumental in CC development by carrying out a systematic study with concentration on specific biomarkers reported in CCSC.

Methods

Hence we used the search engine PubMed using the Mesh terms cervical cancer etiopathogenesis; human papilloma virus role; its types; cancer stem cells; biomarkers of cervical cancer stem cells (CCSC's) ; role of microRNA's.

Results and Discussion

We found a total of 580 articles out of which we selected 50 articles for this review. No meta-analysis was done.

Role of HPV as a crucial factor in CC development

HPV comprises of double stranded and circularized DNA that contains noncoding, long control regions and 8 open reading frames which encode viral proteins located in the ''early'' and ''late'' regions [8,9]. The early stages of HPV DNA replication take place in proliferative basal epithelial cells, giving rise to low viral copy numbers [8]. But the progeny of basal cells replicates and moves into the spinous cell layer where viruses mature; allowing the expression of late capsid proteins and the release of infectious virions within desquamated cells [8].

Viral proteins-role

Early HPV genes are responsible for the synthesis of proteins associated with replication and maintenance of the viral genome. Additionally, late genes encode proteins associated with the formation of the viral coat.

E1, a DNA helicase is needed for viral multiplication and thus maintenance of the viral genome as a multicopy episomy in the nucleus of cells and can arrange protein –protein and protein-nucleic acid interactions. In this sense, p80, human SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member1, histone H1, E1 binding protein and p56, are essential in order to link the DNA replication network of the host cell with the replication origin of the virus. With the role of E1 serving in viral DNA replication with its immediate interaction with the host machinery, there has been proposition with regards to its playing a role in CC development [10].

E2 is believed to be a central transcriptional regulator of the papilloma virus since it interacts with E1 at the onset of DNA repli-

cation, and since this protein scatters the viral episomal genomes at the time of division of infected cells. E2 mediates the interactions between the viral genome and chromatin adapter proteins at the point of mitotic division and indirectly regulates transcription of E6 and E7 producing various effects of the cell cycle which affect proliferation, differentiation, apoptosis and senescence [11].

E4 is associated with successful virus release and can also be used as a biomarker of active HPV infections. E4 is present between the early replication of HPV genes, however, this protein is also expressed during the late stages of the infections [12].

The E5 protein presents oncogenic activities in cultured cells and animals, and it is thought to play an important role in the productive virus cycle. The E5 protein has also been documented to modulate the activity of cellular proteins, interacting with targets like Bax or NFκB, that induces cell proliferation, apoptosis and senescence [13].

E6 is expressed when transformed cells migrate to the spinous cell layer. E6 stimulates protein synthesis by increasing translation through rapamycin (mTOR) complex1, that increases the 5'mRNA cap translation initiation complex. Loss of E6 caused poor maintenance of the HPV genome in view of failure in p53 degradation was documented. E6 avoids apoptotic responses by binding to procaspase-8 and can inhibit the interferon responses by down regulating multiple interferon response genes [14].

E7 stimulates HPV viral replication by reprogramming the cellular environment, together with E6 it induces a potent transformation activity in the host cells. The influence of E7 is seen in multiple cellular processes that includes viral replication, transformation, cell cycle and cell death, via direct or indirect interactions with a large number of proteins [48-52]. E6 and E7stimulate the degradation of p53 tumor suppressor protein through the ubiquitin –proteasome pathway, triggering uncontrolled proliferation of the infected cell population [15].

L1 protein is made up of the isocahedral surface of the HPV virions and it is a 1st part of contact between host cells and the virus. Flexibility is present in L1 to release the viral genome into a new target cell; but assembly of new virions takes place only in fully differentiated karatinocytes which are ready to flake [16]. Moreover L2 also takes part in papilloma virus assembly that starts the infection process. L2 helps HPV DNA encapsulation and it is an important part of the late-stage proteins. Thus, L1 and L2 proteins take part in assembly of virions along with initial events of the infection, because of which they might be utilized as potential vaccine antigens [8].

CSC's

Definition of cell got redefined. At present workers identify the presence of a group of cells that have the ability to renew with high plasticity. SC's differentiate into the most correct lineage, based on the stimuli which they may get from the surrounding microenvironment. Besides self-renewals can develop specialized cells that have limited proliferative ability [17].

This subset of cells role is in maintenance of tissue homeostasis, at the time of daily turnover and regenerate tissue injuries [18]. Once human SC's were discovered, even in adults, emergence of 2 challenges has taken place) Development of laboratory protocols which allow the isolation of SC's in enough quantities and ii) getting insight into the mechanisms which define the fate of these cells [7, 19]. The identification and isolation of SC's have been done utilizing molecular biomarkers that comprise of differentially expressed proteins [5]. But, a constant change takes place in the expression amounts of these biomarkers, based on the environmental conditions, especially in *in vitro* cultures [19,20]. Malignant SC's are known as CSC's; these cells possess some qualities that are similar to normal SC's like self renewal, differentiation, high telomerase expression, evading apoptosis and the capacity to migrate [21]. These CSC's can also transport substances like drugs throughout the membrane, utilizing the membrane protein transport protein adenosine triphosphate –binding cassette subfamily G member2 (ABCG2), that=>the recurrence of the disease in patients following surgery and chemotherapy [5-7].

Origin of CSC's-theories

With the similarities in between SCs and CSC's, different theories are present as per how CSC's originate. DNA mutations might affect somatic cells and SC's and develop CSC's with great tumorigenic activity. Other probability as per DNA mutations which target stemness genes in malignant progenitor into CSC [5,6]. But the possibility is present that CSC's might be present in a dormant state till the initiation of carcinogenesis [5,6].

Metastasis and CSC

CSC's are responsible for helping in cancer metastases in view of their migratory and invasion ability to reach distant organs. Earlier studies have shown that tumor associated CSC's are related to adverse outcomes and high rate of metastasis [22]. EMT is thought to be the initial source of metastatic cells. Transcription factors like SNAI 1, SNAI2, TWIST and BMII polycomb ring finger proto-oncogene (BMII), that are expressed in great quantities in CSC's is behind this transformation of the cell. Loss of epithelial adhesion and apical-basal polarity, gets induced by EMT, that allows the release of transformed cells having properties of CSC into the circulatory system [23]. Figure 1 shows the cells that are undergoing EMT, =>metastasis.

Figure 1

Biomarkers of CCSC's

For CSC's particular markers like CD44, CD90, CD133, CD271, epithelial –cell adhesion molecule and aldehyde dehydrogenase 1 (ALDH1). But no set of universal biomarkers exist for identification and isolation of CSC's [7, 20-24]. Thus the main way to study CSC's is via the ''side population'' (SP) that is a small subpopulation within the tumor mass present in some cases (up to 20%) [25]. CSC like characteristics are present in SP, like the capacity to initiate tumor synthesis, resistance development for chemotherapeutic drugs and its potential as a predictor of patient outcome. Hence the SP might be another source to study CSCs that possess unknown markers [25]. Evaluation on the basis the capacity of the cells to efflux the fluorescent dye Hoechst and gives a system to identify multipotent SC's. Also the spheroid cell formation assay may also be utilized in view of its basis on the ability of CSCs to grow and form spheres in non-adherent environment. The main focus is to evaluate and compare the expression of surface markers through fluorescent-activated cell sorting (FACS), confocal microscopy, immunohistochemistry, reverse transcription (RT-quantitative PCR and the isolation of CSCs for tumorigenic efficiency tests and tumor subpopulation analyses in animal models [7,20].

Thus, CCSC populations exhibit specific expression profiles along with cell surface markers which (on tumor tissues in patients) make their isolation possible *in vitro* along with *in vivo*, and their examination, and research into the progression of cancer might assist in developing double therapies that are directed towards specific targets of the CCSC possible in the coming future. Of the main proteins expressed in CCSC are cytokeratin (CK) and CDs. CKs-5,-8,-13,-17.-18 and -19 are proteins expressed in reserve cells and the immature squamous metaplastic cells of the cervix. CK 19 was introduced by Wang., *et al*. [26], where it was shown that the expression of CK19 in CC was much >than that seen in patients having benign lesions. Additionally, >levels of CK19 were proved by RT-PCR in the CCSCs of sentinel lymph nodes from patients with CC [26] CK 8 and CK17 were examined by Ikeda., *et al*. [27], using immunohistochemistry on the tissues of the patients with different grades of CIN and CC. Hence they concluded that CK 8 and CK17 were expressed by CIN and CC tissues, and that CK17 was related to metastatic processes and the development of highly malignant diseases [27]. Hence CK17 and CK19 might be taken to be biomarkers of CCSCs through the cultivation of CC clinical samples [28]. Further evaluation of main along with 'side population'' using Hoechst 33342 dye, flow cytometry, sorting method, along with tumor formation in nude mice showed that the 'side population'' presented > tumorigenicity and CSC properties [28].

CD44 and CD 133 proteins have been broadly accepted as general markers of CSC markers in multiple types of tumors. CD44 and CD 133 transmembrane glycoproteins take part in normal cellular processes [13] and further in cancer cell migration, aggregation and tumor development [5,6,23]. Thus these glycoproteins can be utilized as surface markers to isolate various kinds of CSCs that includes breast, prostate and pancreas, colorectal, gastric and CC [5,23,29]. Conversely, CD 49f is a highly expressed protein in CCSC and it has advantage in its identification and isolation.

Evaluation of particular surface markers in sphere cells was done by Lopez., *et al*. [24] that were originated from HeLa, SiHa, Ca Sk i and C-41 cells times grown at low density (1000cells/ml) in serum –free medium and found an increase in CD49f and CD 133 positive cells as compared with the monolayer cells.

Typical phenotypes of putative CCSC were revealed by Ortiz-Sanchez., *et al*. [22], that included CD49f+, CK17, p63+, AII+ and ALDH; the sphere culture showed a stemness state, specified by the presence of OCT4, NANOG and β-catenin. Additionally, it was seen that the presence of CD49f and AII was related with the probability that HR-HPV might infect normal cervical cells (SC's. They also showed the high tumorigenic activity of $ALDH^{bright}$ cells as compared to $ALDH^{low}$ cells.

Role of viral oncoprotein E6 in SC signaling and maintenance of stemness in CC was shown by Tyagi., *et al*. [30]. CSCs which expressed particular set of phenotypic markers like CD49f, ABCG2, CD71, and CD133 were removed from primary cervical tumours and cancer derived cell lines grown as spheres. Additionally, transcripts of self-renewal and stemness markers that included OCT4, SOX2, NANOG and Leucine –rich repeat repeat–containing G protein coupled receptor (LGR1) and CD133 were found, besides overexpression of E6, Hes family bHLH transcription factor1, a protein in increasing self renewal characteristics and the capacity of tumor sphere formation [30].

A correlation between CCSC markers in patients tissue samples and the predictability of CIN prognosis was documented by Hou., *et al*. [31]. They evaluated paraffin-embedded surgical samples via IHC, snap frozen CC samples and normal cervical samples utilizing RT-PCR for CD49f, SOX2, ALDH1and musashi-RNA binding protein 1 (MSII). This way patients having tumors classified as high MSII and low CD49f expression had the worst prognosis, while tumors without MSII and CD49f upregulation had the best prognosis. For the 1st time these authors documented, clinical proof regarding CCSC markers associated with prognosis of patients with CC.

More proteins that are differentially expressed in CCSC are NANOG, Nucleostemin (NS), MSII, TWIST, nestin, ALDH1, BMII, piwi like RNA-mediated gene silencing (PIWIL2), TIMP metallopeptidase inhibitor 4 (TIMP4), LGR5, OCT and SOX.

Role of NANOG, NS, and MSII in carcinogenesis of cervix and progression to CC was documented by Ye., *et al*. [32], by doing IHC analysis of 235 paraffin embedded samples with normal cervical epithelia, CIN1, -II and –III; and CC. Staging of CINs was done like this: I, low grade lesion with mildly atypical cellular changes in the lower third of the epithelium (mild dysplasia); CIN II, high grade lesions with moderate atypical cellular changes confined to the basal two-third of the epithelium (moderate dysplasia); CIN III, high grade leasions with severe atypical cellular changes encompassing the full thickness of the epithelium (severe dysplasia). They found high levels of expression of the3 proteins in CC, CIN-II and-III and low expression levels in CINI and normal cervical epithelia. But no correlation was found among NANOG, NS, and MSII expression levels and the prognosis of CC. Additionally, NANOG, NS, and MSII also have a key role in the carcinogenesis of glioma, liver, gastric and other types of cancer [32].

Activation of Wnt/β-catenin and Akt signaling pathways in TWIST overexpressing cells which had CSC Properties like tumor sphere formation and ALDH1 and CD44 expression levels was observed by Li and Zhou 2011 [33]. They documented that TWIST is an indicator of morphological changes related to EMT. Additionally the spheroid cells gained expression of human actin – α cardiac muscle 1 (also called α-smooth muscle actin) and Vimentin mesenchymal markers. On functional analysis it was shown that spheroid cells are more resistant than monolayer cells to paclitaxel [33]. Conversely, knockdown of β-catenin expression by small-interfering-RNA transfection and Akt signaling pathway inhibition by the PI3K/Akt inhibitor wortmannin suppressed the expression of CD44 [33].

The role of Nestin in CIN and CC was studied by Sato., *et al*. [34], utilizing in situ hybridization and IHC evaluation. Normally expression of nestin occurs in brain, but it was also found in CSC's, various metastasized carcinomas along with in kinds of cancer that had a bad prognosis predicted. They evaluated tissues from 26 subjects with CIN and 55of CC. They observed low nestin expression levels in basal squamous epithelium of CIN I; but in CINII nestin was seen in 65% of subjects and in CINIII most of subjects had nestin localized in squamous epithelium.

Moreover, Nestin was observed in all of invasive CC samples. Additionally, they analyzed effects of overexpression of Nestin

through transfection of ME-180-cells; a metastatic cancer cell line derived from the cervix. This overexpression of Nestin stimulated a > ability to form spheres, develop a CD44high/CD24low pattern related to the biomarkers observed in breast CSCs, enhanced NANOG, OCT4 and ALDH1 expression. Thus, they concluded that Nestin might be related to the progression of CIN to CC and could be related in the control of CSC's in view of its capacity to induce sphere formation *in vitro* [34].

Normally ALDH1 represents a cytosolic isoenzyme that controls oxidation of retinol to form retinoic acid. Great levels of ALDH1 in CCSC's were seen by Liu and Zheng [35]. High ALDH1 activity levels were seen in a subpopulation of subjects with CC having a high ability to get self-renewed, >differentiation potential, high tumorigenicity, as seen in CSCs. They utilized FACS and functional assays in xenografted NOD/SCID mice, along with as cultures of CC cells that expressed high and low levels of ALDH1 in serum free media to form tumor sphere cells. Thus they concluded that ALDH1 might work as a CCSC marker. In their study cells possessing high ALDH1 activity had cisplatin resistance and formed a >expression of NANOG, OCT4, Kruppel like factor4 (KLF4) and BMII.

SOX 2 positive CCcells had all the properties of CSCs that were self renewed, >differentiation and tumor stimulating characteristics was shown by Liu., *et al*. [36]. Further SOX2 –positive cervical cells enhanced the levels of OCT4, BMII and ALDH1 stemness markers, along with in form of Vimentin, SNAII and β-catenin (mesenchymal SC markers. Because of this they concluded that SOX2 might be a key factor in self renewal, pluripotency and further a stemness factor required for SC's and CCSC s differentiation [36].

Role of TIMP4 in the stemness of CC4 was reported by Lizzaraga., *et al*. [37]. TIMP4 is a tissue inhibitor which has been overexpressed in a lot of cancer cell lines and in nude mice, to examine its role in carcinogenesis. Their observations showed a rapid tumor development in nude mice which overexpressed TIMP4 in CC4cells. Activation of NFκB signaling pathway and the increasing CSC population caused a > expression of pluripotency markers in the form of OCT3/4 and SOX 2, The EMT markers SNAII, and Vimentin along with the drug transporter markers ABCG1 and ABCG2 [37].

Role of PIWIL2 in CC tumorigenesis was shown by Feng., *et al*. [38]. PIWIL2 expression was present in the HPV+CC cell lines HeLa, SiHa, Ca Sk I, and was not detectable in HPV Cancer line C33A. On knockdown of PIWIL2 with the use of short hairpin RNA in HeLa, and SiHa cells reduced the tumorigenic, proliferation and chemo resistant capability of these cells. Conversely over expression of PIWIL2 in Ha Cat cells activated tumor-initiating capacity and cMyc, KLF4, NANOG, OCT4 and SOX 2 cell reprogramming factors got upregulated [38]. They further showed that reactivation of PIWIL2 by E6 and E7 oncoproteins is necessary in the transformation of cervical epithelial cells into CSCs. PIWIL2 was markedly expressed in CINII, CINIII, and CC, but only had low expression in CINI and normal epithelial cells. Further PIWIL2 suppressed the expression of P53 and P21 in CC cell lines, inducing cervical carcinogenesis [38].

The main role of LGR5 in CC for activation of Wnt/β-catenin signaling pathway was earlier documented by Cao., *et al*. [39]. Further they showed the function of LGR5 in CCSCs through overexpressing and silencing its action in CC cell lines. Hence they demonstrated that overexpression of LGR5 induces CSC properties that includes tumor sphere formation, enhanced tumorigenic ability *in vivo*, chemoresistance to cisplatin, increased cell migration and invasion, and upregulate the expression of stem-cell associated transcription factors *in vitro*. Overexpression of LGR5 in HeLa, and SiHa cells was shown to be associated with > expression of BMII, KLF4, NANOG, OCT4, as compared to control or silenced cells [39]. Summarized in figure 1 the main factors responsible for progression of CC, besides changed microbiota population, throughout CC development and progression.

Signaling pathway of CSCs

Hedgehog (Hh), Notch, Wnt, NFκB and PI3K/Akt/mTOR signaling pathways get shared by SCs and CSCs. Hh represents a necessary required for self-renewal and cell fate, being associated with tumorigenesis, formation and progression of some cancer kinds, along with maintenance of CSCs, Stemness in CSCs is driven by the Hh signaling pathways through the genetic control of OCT4 SOX 2, and BMII [40]. In association with CC, Hh is related to bad results in patients that received irradiation with proof available that Hh, causes repopulation of cervical cells after chemoradiation.

Proliferation, maintenance of Stem cell, specification of cell fate, differentiation along with angiogenesis is regulated by the Notch

signaling pathway. Additionally, this Notch signaling pathway controls cell-cell communication via transmembrane ligands as well as receptors. The canonical pathway represents 5 canonical Notch ligands namely Δ-like canonical Notch ligand (DLL), DLL3, DLL4, Jagged 1 and Jagged 2, along with the 4 receptor paralogs. Different Notch receptors and ligands get expressed via cancer cells, further the non-canonical pathway might also have the same role in cancer. Hence both pathways may control a variety of tumor –associated cells along with CSCs or immune cells. Moreover, expression of various Notch paralogs differs between various kinds of tumor. Notch might show a variety of functions in CC, based on the progression of the disease: I) maintenance of immature epithelium by preventing terminal differention, ii) increasing NOTCH expression during the progress of CIN into CC, iii) regulation of Notch signaling pathway through E6 in CC cell lines and iv) silencing Jagged 1in Ca Sa ki cells inhibits its tumorigenic ability [41].

Wnt signaling pathway control cell Proliferation and different ion at the time of embryogenesis. Through Wnt signaling cascades the canonical pathway is the one most examined for its role in cancer formation. Multiple publications suggest a contribution of CSC in the maintenance of these cells via the Wnt/β-catenin signaling pathway. Apoptosis gets induced in CC along with inhibition of tumor growth occurs on suppression of Wnt signaling pathway. Conversely over activation of Wnt/β-catenin signaling pathway is related to cervical tumorigenesis with HPV infection [42]. In the basal layer, Wnt ligands are needed for maintaining the undifferentiated state of SC's. Most of genetic mutations in colorectal cancer activate the Wnt signaling pathway, and CSCs are most susceptible to transformation by these mutations. NFκB plays a key role in HPV infected cells. This signaling pathway is implicated in cancer formation through various oncogenic genes [43]. NFκB signaling pathway possesses 2 routes i) the canonical pathway, which depends on the inhibitor of NFκB kinase complex (IκB) and ii) the non-canonical pathway, that gets activated when the homodimer IκB, inhibitor of nuclear kappa –B kinase subunit –α (IKKα) is phosphorylated. IKK-α is part of the IκB complex, and is related with the growth, metastases and stemness of various kinds of cancer [44].

In the PI3K, /Akt/mTOR signaling pathways PI3K and mTOR play a key role in cell proliferation, angiogenesis, metabolism, differentiation and survival [45]. This signaling pathway gets mostly activated if mTOR does not get regulated in a correct way in cancer conditions. Over expression of PI3K has been found in ovarian cancer and CC [46]. PI3K Akt/ mTOR is a necessary signaling pathways for control of self-renewal and the maintenance of stemness in SCs and CSCs. Function of CSCs, is well known in prostate cancer [21,47], though the mechanism via which PI3K, /Akt/mTOR signaling controls CSCs is not known.

Role of micro RNA (miRNA)

Further Wang., *et al*. found that miR23b was under expressed in CCSC's to maintain high amounts of ALDH1. Introduction of miR23b into cervical cancer cells could alter stemness and cisplatin sensitivity after studyingALDH1 associated miR's in their isolated tumor spheres (TCs), concluding miR23b played a crucial role in maintaining stemness of CCSC's and thus can be used to form therapeutic target to fight CC in a better way [48]. Dong., *et al.* [49], studying the role of miR146a on CSC's found that TCs enriched from the HeLa cell line showed high ALDH activity and miR146a was upregulated in differentiated TC's. Further miR146a inhibitor increased colony formation and cell invasion in TC's while miR146a mimics showed opposite roles. Inverse relation was observed in TC's between miR146a and VEGF expression and the luciferase reporter assay showed that VEGF was a direct target of miR146a. Inhibition of VEGF reversed the effects of mi146a inhibitor on TC's. The activated CDC2/PAK1 signaling was correlated with TCs tumorigenesis and invasion. Moreover, miR146a inhibitor treated TC's promoted tumor growth in nude nice. In total these results pointed that miR146a modulated TC's tumor formation and invasion, that was related to VEGF/ CDC2/PAK1 signaling. Thus this study gave a good understanding for developing newer therapeutic methods for CC [49].

Conclusions

The study of Molecular pathogenesis of CC has been the effort of lot researchers along with finding how viral infections progression contribute to the development of invasive cancer. Although most studies have tried to confirm that CCs aetiology is HPV infection, but current researchers have tried to work at the molecular level, which factors and changes affect the stemness and formation of CC. This way a lot of proof has found CCSCs, being the novel, fundamental and strategic critical factor that needs attention in cancer formation, chemotherapy resistance and regression of cancer. The hypothesis that is most recognized is that transformation of SC's through HPV infection using their E6 and E7 oncoproteins whose

normal activity is the maintenance of stemness in healthy cells. But KLF4, NANOG, OCT4 and SOX and Nestin proteins also pay a role in maintenance of stemness of CSCs.

Other proteins responsible for maintenance are ABCG2, SNAII, Vimentin and LGR5, though this is not their initial main role. Thus, there is a complex network in the formation of CC along with its progression. Hence looking for biomarkers for every stage of disease is necessary to get insight in the development of CC along with development and application of novel treatments along with getting accurate diagnosis.

Right now a lot of workers are trying to find newer targets responsible for the stemness of CC. Hence genes, proteins and signaling pathways are under study. Some potential candidates to get this aim in CCSC formation, CD44, CD133 and CD49f might be attractive targets to direct the treatment strategies and blockade of Hedgehog, PI3K, /Akt/mTOR, Wnt or Notch signaling pathways in CCSC. Further targeting miR 23b and 146a might provide a newer target for development of novel strategies for CC treatment.

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Volume 3 Issue 11 November 2019