REVIEW

Role of Cytokines in Genesis, Progression and Prognosis of Cervical Cancer

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Abstract

Cytokine research is currently at the forefront in cancer research. Deciphering the functions of these multiple small molecules, discovered within the cell and in intercellular spaces, with their abundance and pleotrophism, was initially a great challenge. Advances in analytical chemistry and molecular biology have made it possible to unravel the pathophysiological functions of these polypeptides/proteins which are called interleukins, chemokines, monokines, lymphokines and growth factors. With more than 5 million women contracting cervical cancer every year this cancer is a major cause of mortality and morbidity the world over, particularly in the developing countries. In more than 95% of cases it is associated with human papilloma virus (HPV) infection which is persistent, particularly in those with a defective immune system. Although preventable, the mere magnitude of prevalence of HPV in the world population makes it a dominating current health hazard. The discovery of cytokine dysregulation in cervical cancer has spurted investigation into the possibility of using them as biomarkers in the early diagnosis of cases at high risk of developing cancer. Their critical role in carcinogenesis and progression of cervical cancer is now being revealed to a great extent. From diagnostics to prognosis, and now with a possible role in therapeutics and prevention of cervical cancer, the cytokines are being evaluated in all anticancer approaches. This review endeavours to capture the essence of the astonishing journey of cytokine research in cervical neoplasia.

Keywords: Cytokine - cervical cancer - carcinogenesis - progression - prognosis

Introduction

There is a marvelous coordinated orchestrated balanced sequence of events that controls normal cell function, cell division and programmed cell death. There is even a more complex physiology that allows repair and restoration of any dysfunction that may occur. When these functions are disturbed there could be dedifferentiation and uncontrolled proliferation that characterizes carcinogenesis. Initially the dysfunction is reversible and later irreversible. Cytokines are functional small peptides which under physiological conditions control the "cell-to-cell communication" within the various body tissues ie paracrine function. Sometimes, when the cell which secretes a cytokine also has the receptors for the same cytokine on its cell membrane, the cytokine may control the function of the very cell from which it originated ie autocrine function, whilst less commonly cytokines spill over into general circulation and can affect distant tissues and organs, ie endocrine function (Platanias, 2007; Chedrese, 2009). Cytokines are also called as interleukins, monokines, lymphokines, chemokines and growth factors. It has been observed that the local tissue or circulating cytokine levels is altered in a number of cancers, including gynecological

cancers (Murooka et al., 2005; Heikkela et al., 2008). Cervical cancer is a huge challenge to the health systems of several countries in the world, affecting the quality of life and loss of work- hours of millions of women in the age group of 40 to 65 years of age whilst more than 50% of them die because of late diagnosis in spite of expensive treatment. Those living with treated cancer usually have a very poor quality of life (Moore et al., 2010; Sankaranarayanan et al., 2010; Takiar et al., 2010; Le Borgne., 2013). This leads to a tremendous waste of human life and painful, expensive treatment modalities like radical hysterectomy, exenterations, radiotherapy or chemotherapy even though it is well demonstrated that cervical cancer is preventable through adoption of HPV vaccination, Pap smear screening and HPV detection tests.

In spite of attempts at mass screening, one of the reasons why large numbers of cervical cancers occur (apart from failure to implement primary prevention), is the massive numbers of women with precancerous conditions, namely Low-Grade Squammous Intraepithelial Lesions (LSIL) and High-Grade Squammous Intraepithelial Lesions (HSIL), who are also detected in screening programmes (Bethesda 2001; Janicek et al., 2001; Sankaranarayanan et al., 2010). Whilst there is progression to invasive

cancer in a small number of cases of LSIL, and a larger proportion of cases with HSIL, many regress or remain arrested (Moscicki et al., 2004, Melnikow et al., 2009). It is therefore neither desirable nor feasible to follow up all these women at frequent intervals with repeated Pap smears/HPV tests. The success of the screening programme in cervical cancer prevention will therefore depend on identifying those precancer cases with a higher risk of progression than the rest and closely following them up so that invasive cancer is prevented.

Recent literature reveals a significant association between dysregulation of some cytokines and the incidence of cervical precancer (LSIL, HSIL), progression from precancer to "*in situ*" cancer, further invasion and also end stage metastasis. Initially cytokines were believed to be messenger molecules in the immune system guiding the leucocytes to the sites of inflammation. However, when dysregulated, they have now been shown to be associated with most neoplastic tissues and may have a role in malignant transformation, proliferation, survival, angiogenesis, invasion and metastasis. There are almost 50 cytokines identified now and some of these or their receptors are significantly altered in carcinogenesis and metastasis of cervical cancer. Cytokines like IL-6, IL-8, IL12, ILR4, VEGF, IL-4, IL-10 etc have been shown to serve as potential biomarkers to assess the risk of invasive cancer and metastasis (Markowska, 2007; Jayshree et al., 2009; Huang et al., 2013). In this review, we have summarized the pathophysiological role of cytokines in the carcinogenesis and progression of cervical cancer.

The major events relating to persistent HPV infection with High Risk HPV types and the cervical carcinogenesis have been reviewed earlier in details (Dutcher et al., 1988; Clerici et al., 1998; Hausen, 2000; Markowska, 2007; Boccardo et al., 2010). Many cytokines are markedly altered in cervical precancer and cancer, more so in advanced cancer with metastasis. Excess of some of these, eg IL-6, IL-17, IL-8 is associated with tumor growth whilst some are associated with inhibition of HPV replication and suppress tumors eg. IL-1, TNF-α, TGF-β, IFN-α, at least in the initial stages.

Structure and Functions in General

Advances in molecular endocrinology and cell signaling pathways have set the foundation for the discovery of cellular pathways of various growth factors and cytokines and their physiological and pathological role in endocrine function and carcinogenesis. Cytokines are small polypeptides or proteins which are secreted by several tissue cell types, usually of the immune system, and have pleotrophic function at the local tissue level or occasionally at systemic level. They control the growth, differentiation or activation of different cell types. They act through cytokine receptors on the cell membrane or soluble plasma or tissue fluid receptors. They are small molecules with molecular weight about 27kDa to 30kDa. More than 50 chemokines or cytokines have been identified alongwith their receptors and putative functions. (Gururaj, 2005; Platanius, 2007).

Cytokines function synergistically and have pleiotropic

and redundant functions. Those involved in cancer biology can be classified as immunostimulating Th1-type cytokines which include Tumor Necrosis Factor-α (TNF-α), interferon-γ (IFN-γ), interleukin 2 (IL-2), and IL-12. They mainly induce cell mediated immunity and work as tumour suppressing cytokines. The Th2-type cytokines are immunoinhibitory and include IL-4, IL-5, IL-6, IL-8, and IL-10, for cell mediated immunity and primarily induce humoral immunity (Clerici et al., 1998; Bais et al., 2005). Th1 cytokines are crucial for inducing an adequate anti-tumor immune response. On the other hand continuous expression of Th1 cytokines can promote the chronic inflammatory process which causes generation of reactive oxygen and nitrogen species. These changes induce DNA damage and make the cells susceptible to neoplasia.

The genetic location of many cytokines, their molecular structures and of their receptors have now been identified. Experimental studies with "knock out mice" have helped in identifying specific functions of several cytokines. Alterations or mutations in these genes can cause or increase susceptibility to monogenic or polygenic disorders like autoimmune disorders, susceptibility to severe infections, or to certain cancers including cervical cancer eg. IL-1-β, IL-4, IL-6, IL-10, TNF-α (Deshpande et al., 2005; Zachary et al., 2007; Castro et al., 2009; Gangawar et al., 2009; Zarogoudilis et al., 2013).

The cytokines like IL-6 were initially detected in experimental animals with cancer (Gelin et al., 1988). One of the earlier important studies on the role of IL-6 in human cancers was carried out by Tabibzadeh et al. (1989). Taking a cue from the earlier studies in experimental animals and in human tissues they studied the immunohistochemical positivity of Interleukin-6 (IL-6) in a number of cancer tissues and normal tissues. A strong immunopositivity with a specific Avidin Biotin Complex (ABC) Assay using polyclonal rabbit antiserum raised against a human IL-6 (rIL-6) was observed in pathology sections from primary squamous cell carcinomas, in adenocarcinomas of mammary, colonic, ovarian, and endometrial origin, in various adenocarcinomas metastatic to lymph nodes, and in soft tissue tumors including leiomyosarcoma and neurofibrosarcoma. Weak or moderate IL-6 immunostaining was also observed in Hodgkin's and non-Hodgkin's lymphomas indicating that most human cancers are positive for increased expression of IL-6.

Cytokines may belong to the 4 types: TNF family, Chemokine family, Interferon family, Hematopoietin family. Growth factors are also included in chemokines and TGFs, IGFs, GMCSF and VEGF are examples of growth factors which influence carcinogenesis, tumor growth as well as metastasis (Tabibzadeh et al., 1989; Platanias, 2007; Sharma M et al., 2012).

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Individual Cytokines Commonly Dysregulated in Cervical Cancer

EGFs

Epidermal Growth Factor and its receptors were amongst the first identified biomarkers of cervical cancer (Soonthornthum et al., 2011; Gadducci et al., 2013). EGF is a mitotic growth factor overexpressed alongwith IGF I and IGF II, which are also overexpressed in cervical cancer. The EGFs are of 7 subtypes and are associated with important physiological functions in various tissues whilst overexpression is associated with some pathologies and cancers including cervical cancer. The major prognostic abnormality has been an overexpression of the EGF receptor EGFR, particularly for survival after radiotherapy.

EGFR

The receptor for EGFs has 4 subtypes in the family and it is a transmembrane tyrosine kinase. EGFR is also known as the HER (Human Epidermal factor Receptor) receptor and is a target for treatment. EGFR/HER family inhibitors, such as gefitinib, erlotinib, cetuximab, lapatinib, trastuzumab, panitumumab, are being evaluated in cervical cancer and are reviewed in depth (Lida et al., 2011; Soonthornthum et al., 2013; Vici et al., 2014). Most of these are injectables (subcutaneous, intramuscular or intravenous), whilst some like erlotinib, are available as oral tablets.

IGFs

Activation of Insuline like growth factor (IGF) pathway is related to several gynecological cancers, particularly endometrial cancer but is also documented in cervical cancer. Growth factors are important mitotic agents and their overexpression leads to hyperplasia or cancer. IGF-1 activation is observed in cervical cancer. Retinoid analogues inhibit the function of the EGF and IGF1 signalling pathways and have been extensively evaluated in cervical cancer chemoprevention clinical trials. The efficacy of oral or local retinoid analogues in cervical cancer chemoprevention after CIN 2 or 3 has developed is of limited clinical value in randomized clinical trials (Helm et al., 2013).

IGFR

The IGF receptor is overexpressed in cervical cancer, hence the blocking antibody to IGFR has been evaluated in advanced cervical cancer with minimal clinical benefit.

IFNs

Interferons are immunoprotective cytokines and have been shown to be defective/deficient in cervical cancer. Human IFNs are of type 1, ie IFN-α, IFN-β or type 2, ie IFN-γ (Parmar and Platanias, 2007). These have all been evaluated in experimental and clinical studies for immune function and also in cervical carcinogenesis, both in limited disease as well as advanced disease (Dutcher et al., 1988; Boccardo, 2010). IFNs act through the Jak-Stat and also through the CBL-Crk signaling pathways. Deficient function because of reduced expression or altered receptors is associated with reduced apoptosis and cell proliferation in several cancers including cervical cancer. There are several subtypes of IFNs and their receptors. IFN gamma can reduce the HPV E6/E7 protein expression (Eckert et al., 1995). Recombinant IFN-α injection therapy has been evaluated in CIN as well as in invasive and in advanced cervical cancer. IFNs may be induced by HPV infection, particularly the E6 and E7 proteins of the High Risk HPV (HR HPV) types. HR HPV infection induces an IFN response and in most cases the infection is controlled and is undetectable within 2 years. However in some cases, particularly with defective IFN function, the HPV infection persists and in a few cases IFN may actually activate HPV transcription and the E6 and E7 proteins. These interfere with the protective action of IFNs at several levels and allow escape of HPV virus from immune degradation or clearance. The persistant HR HPV infection is prone to cervical carcinogenesis. IFN injectable therapy has been studied in cervical advanced cervical cancer cases but has not been very encouraging (Liu et al., 2004; Tirone et al., 2010; Misson et al., 2011).

IFN therapy has also been also been used in cases with cervical intraepithelial neoplasia to cure or arrest of the cervical lesion (Spitzbart et al., 2000; Misson et al., 2009; Misson et al., 2011, Machado et al., 2012). In a study in which circulating levels of several cytokines were measured before and after therapy of CIN 3 cases with intralesional IFN therapy Misson et al. (2011) observed that responders exhibited elevated levels of IL-12 in responders.

TNF

Tumor necrosis factor (TNF, cachexin, TNF-α) is a cytokine with 233 aminoacids and a molecular weight of 25.6 kDa. It is secreted principally by activated macrophages in response to acute inflammation. Circulating levels of TNF are increased in fever, sepsis, cancer, Alzheimer's disease, depression, and irritable bowel syndrome. It is secreted in large amounts by IL-1. Its actions in several tissues in the body are in relation to IL-1 and IL-6. It was found to induce apoptosis and tumor cell necrosis in several types of cancer cell lines hence the nomenclature. However it has several actions in the body eg. insulin resistance and obesity. TNFα levels have been found to be increased in local tissue specimens from cervical cancer and hence blocking antibodies have found therapeutic application. TNF polymorphism is associated with increased risk of cervical cancer (Liu et al., 2012). Very interestingly TNF-α gene polymorphism can increase or decrease the susceptibility to cervical cancer depending

on whether it is TNF-α-238A allele or TNF-α-308G>A which is altered (Pan et al., 2012).

TRAIL or TNF-Related-Apoptosis-Inducing factor has been investigated for cervical cancer therapeutics in combination therapies (Vici et al., 2014).

TGF-β

Transforming Growth Factor (TGF-β) is one of the earlier chemokines to be found to be associated with the malignant transformation of cells. It is a 25 kDa protein with 390 aminoacids in TGF-β 1. It controls the growth, proliferation and differentiation of most cell types through immune modulation. Tumor macrophages produce TGF-β and IL-10 in large amounts leading to both uncontrolled mitosis and immune escape phenomenon which favors tumor survival. In normal cells TGF-β has a growth inhibitory effect which requires an intact P38 pathway. However in most cancers the TGF-β pathway activates growth signals, causes escape from apoptosis, and also promotes tissue invasion and metastasis. There are TGFRs on the cells which secrete TGF and hence there is autocrine signaling of TGF-β secretory pathway when the tumor develops. There are about 30 members in this family and isoforms which bind to the TGF-β receptor (TGFR). Both mutations or polymorphisms of TGF-β and TGFR are associated with cancer risk. TGF-β acts through SMAD and DAXX pathways in normal cells to induce apoptosis. In cancer tissue it increases IL-10 and expression of MMP-1 and MMP-9. It also increases the expression of VEGF and is involved in metastasis. This pathway therefore is a major target for development of inhibitors for TGF blockade (Platanias, 2007; Vici et al., 2014).

IL-1 α/β

IL-1 was the first identified soluble factor from macrophages identified with paracrine proinflammatory activity. Later it was found that it is also expressed by several other immune cells including the lymphocytes. It increases the adhesion factors and assists the migration of leukocytes to the site of infection. There are a total of 11 members in the IL-1 family with similar biofunctions. These are secreted as pro-cytokines and are subsequently cleaved to form the smaller cytokine units of about 25 to 33 kDa depending on the exact product. Increased levels of IL-1 α/β and IL-10 were observed in cervicovaginal secretions of women with CIN, HPV and HIV indicating the adverse effect of Sexually transmitted infections on cytokine profile (Mhatre et al., 2012). Both IL-1 α/β act via IL-1RI and the polymorphism of IL-1 as well as its receptor are associated with risk of cancer. Immunotherapy with IL-1 is reviewed by Vici et al. (2014)

IL-2

IL-2 is a glycoprotein with 153 Aminoacids and a molecular weight of 15 kD. It is produced by antigen-activated T lymphocytes including the CD4+andCD8+ lymphocytes, and other immune cells. It has a paracrine effect on various immune cells including lymphocytes. It activates the tyrosine kinases and phosphorylation of several proteins resulting also in changes in the JAK/STAT signaling pathways and Src kinases (Platanias, 2007). IL-2 levels were increased in peripheral blood lymphocyte culture supernatants in women with HPV peptides exposure *in vitro* and were maximum in women with normal Pap smears than in women with CIN or cervical cancer (Tsukui et al., 1996). Injectable formulation of IL-2, as an immunoprotective cytokine has been studied in experimental animals for treatment of precancer or CIN. Clinical trials, Phase 1-3, have been carried out with reasonable success in CIN and in advanced cases of cervical Cancer. It can be used in combination with HPV vaccine (Dutcher et al., 1988; Liu et al, 2004). Preliminary results have been encouraging in CIN 2 and 3. IL-2 receptors have 3 distinct subunits-alpha, beta and gamma chains. The exact mechanism of action of IL-2 is not understood well, but it is known to modulate several immune functions and cytokines.

IL-4

IL-4 is a glycosylated protein with 18 kDa molecular weight, and is secreted by activated T lymphocytes, basophils and mast cells. It is also known as B cell Stimulatory Factor (BSF). Under physiological conditions it has immune defense mechanism and stimulates IgG secretion. It is important in asthma and allergies. It has pleotrophic action, and can either promote or inhibit tumour growth depending on the microenvironment within the tumour. IL-4 levels in cervical tissue and vaginal washings were increased in CIN and cervical cancer. The highest production of IL-4 and IL-10 was detected in patients with HPV infection that had extended beyond the genital tract (Olver et al., 2007; Yang et al., 2007; Peghini et al., 2012). IL-4 Rp1/Rp2 gene polymorphism is also reported to be associated with an increased risk of cervical cancer (Shekari et al., 2012). In a large meta-analysis of IL-4 R (Interleukin-4 Receptor) polymorphism Wang et al. (2012) observed reduced risk of cervical cancer in Q576R G allele carriers.

Interleukin-6

This glycoprotein has 184 amino acids and has a molecular weight of 26 kiloDaltons (kDa). Its physiological binding to the receptor signals the JAK1, JAK3 and TYK2 which phosphorylate and activate the STAT3 and STAT1 which translocate to the nucleus and regulate several genes. It plays a role in immune defence, differentiation and maturation of B cells into plasma cells, maturation of megakaryocytes. It is one of the major physiological inducers of acute phase proteins. However under pathological conditions it can have a proinflammatory and carcinogenic potential.This pleotrophic multifunctional interleukin is the most clinically relevant and studied cytokine, both in chronic inflammation and in several types of cancer, including cervical cancer. High levels are reported in lung, colorectal, breast, brain, liver, bone and gynecological cancers. (Kawano et al., 1988; Murooka et al., 2005; Heikkela et al., 2008; Zarogoulidis et al., 2013). Elevated circulating levels of serum IL-6 are associated with fever, cachexia, depression, rheumatoid arthritis and obesity. Tabibzadeh et al have reported high tissue levels with semiquantitative immunostaining in several cancers.

McIntosh et al (1989) showed the high levels of circulating IL-6 in tumor bearing mice. The same group (Mule et al., 1990) reported antitumor activity of recombinant human IL-6 in tumor bearing mice, thus showing variable action of IL-6 depending on experimental conditions. High levels of IL-6 have been reported in cervicovaginal washings and in serum in cases of Intraepithelial neoplasia and cancer of the cervix (Tjiong et al., 1999; Tavares-Murta et al., 2008). Tjiong et al studied cytokine concentrations in women with cervical cancer, with CIN, and in healthy controls. The median IL-6 concentration in cervicovaginal fluid was 171pg/ml in cervical cancer cases *vs* 22pg/ml in CIN *vs* <2pg/ml in controls. Similarly IL-8 levels were also increased in women with cancer or CIN. No relation was found between cytokine levels and CIN grade or between cytokine levels and the inflammatory infiltrate scored by histological analysis.

Bustamam et al. (2008) have reported on the cervical carcinogenesis with cervical intraepithelial neoplasia (CIN) which preceded cancer in a mouse model. They induced carcinogenesis in female offsprings of pregnant mice exposed to Di-Ethyl- Stilbestrol (DES), a synthetic estrogen. They followed up various groups through CIN, cancer and metastasis. They demonstrated a significal correlation between local cervicouterine and serum levels of IL-6 with increasing grades of CIN and metastasis.

Apart from the inhibition of apoptosis, IL-6 promotes cervical cancer tumor growth also by increasing neo-angiogenesis by increased VEGF via STAT3 pathway, thus increasing angiogenesis (Wei et al., 2003). IL-1α and TNF-α can stimulate IL-6 gene expression by the keratinocytes and cancer cells. IL-6 also has an autocrine stimulation during carcinogenesis.

We have been interested in cervical cancer screening and prevention over a decade and at our centre the Reverse Pharmacology path (Patwardhan et al., 2010; Godse et al., 2011) is used for developing effective remedies based on traditional medicine. Previous work at our centre has established the anticancer activity of various types of extracts of Haridra or turmeric as it is commonly known (*Curcuma longa* Linn) in various experimental studies and clinical studies in Oral Submucous Fibrosis and in healthy volunteers (Hastak et al., 1997; Joshi et al., 2003). We studied the preventive activity of a supercritical extract of turmeric oil in women with persistent Low-Grade Squammous Intraepithelial Lesion (LSIL) in Pap smears (Joshi et al., 2010). Circulating levels of IL-6 were high in women with abnormal Pap smears (ASCUS, LSIL, and HSIL) as compared to those with inflammatory or negative smears. Furthermore, we observed a significant decline in serum IL-6 levels from a mean of 248±156 (SEM) to 27.7±10.5 (SEM) pg/ml when women with persistent LSIL were treated with a Turmeric oil extract orally for 3 months (Paradkar et al., 2010; Joshi et al., 2011). This was associated with arrest of the LSIL features in Pap smear or regression to ASCUS, or Negative pattern. Circulating levels of IL-6 therefore can potentially be used as an additional noninvasive biomarker in cervical cancer chemoprevention trials.

Similarly other medicinal plant extracts have been shown to have significant anticancer activity in cervical cancer cell lines (Dwivedi et al., 2011; Palasap et al., 2014). Roy and Mukherjee (2014) recently demonstrated the reversal of resistance to cisplatin by adding curcumin in cisplatin resistance SiHa cervical cancer cell lines. Basu et al. (2013) evaluated the positive therapeutic response of HPV infection in 289 HPV positive women with use of curcumin containing polyherbal vaginal cream as well as curcumin containing vaginal capsules for a period of 1 month. However they have not correlated response with cytokine levels. Curcumin independently has been shown to inhibit IL-6 activity and the NF-kB pathway in cancer cell lines and has recently, in a large clinical study, shown improvement in cytokine profile and quality of life of patients with solid tumours (Panahi et al., 2014).

The mechanism of tumor promoting action of IL-6 has been studied in depth by earlier authors. IL-6 is produced by lymphocytes, macrophages, endothelial cells and by several cancer cell types. It has proinflammatory actions and is associated with pyrexia, anorexia, Acute Phase Proteins, C-reactive protein. It acts on various cell types through the IL-6 receptor. It functions through the NFkB pathway which is shown to be activated in majority of cancers including cervical cancer. This results in blocking of apoptosis and uncontrolled growth of the cancer cells. It acts through the janus kinase-signal transducer and activator of transcription-zinc finger protein 1-2 signaling pathway. It promotes neoangiogenesis and hence high local or circulating levels of IL-6 are associated with invasive cervical cancer and metastasis. IL-6 levels are also increased in other diseases like coronary heart disease, fevers, obesity, depression etc. In addition, increased levels of inteleukin-6 have been found to increase the production of collagen and a-actin which induce interstitial lung disease. IL-6 antibody is available in an injectable form for the treatment of rheumatoid arthritis (Zarogoulidis et al., 2013; Vici et al., 2014).

Interleukin-6 Receptor (IL-6R) and Signal Transduction

IL-6 binds to a membrane bound heterodimeric receptor with 1 unit (an 80 kDa membrane bound glycoprotein), which cognizes and binds to the IL-6 ligand, and the other unit is the signal transducting unit of the gp130 family (130 kDA transmembrane proetein). This leads to the activation of JAK kinases and further to other signaling proteins like MAPK, STAT etc. The 80kDa IL-R unit can be cleaved and becomes available as the soluble receptor as well which can bind to the ligand in extracellular fluid and then it migrates to the cell bound gp130 component (Jones et al., 2001; Smola-Hess et al., 2001; Murooka et al., 2005; Zarogoulidis et al., 2013). There is further downstream activation of NFkB and inhibition of apoptosis as well as stimulation of tumor cell growth which characterizes the critical role of IL-6 in cervical cancer as well as breast and lung cancer. The Notch-3 pathway is also activated by IL-6 in cervical cancer (Zachary et al., 2007). TNF-induced changes in IL-6 mRNA, STAT3, and c-myc mRNA are through the activation of NFkB (Kirillova et al., 1999).

Smola-Hess et al. (2001) and Jones SA et al. (2001)

have shown that the soluble receptor of IL-6 (slRIL-6) is an important component of physiology and disease and HPV-18 LCR related cervical cancer growth. Smola-Hess et al (2001), in cancer cell lines, have shown that there is loss of membrane receptor in cervical cancer cells and as a result, there is no auto-inhibition of IL-6 secretion so the transformed malignant cells continue to secrete large quantities of IL-6. The addition of the soluble receptor in the tissue culture restores IL-6 signalling, and stimulates the NFkB system at the same time inhibiting apoptosis, thus promoting uninhibited proliferation. Recently Pahne-Zeppenfeld et al. (2013) have confirmed that IL-6 is the crucial cytokine produced by cervical cancer cells activating the NFkB pathway and influencing the MMP-1 and Dendritic cell migration thus promoting tumor growth and metastasis.

VEGF

Vascular Endothelial Growth Factor (VEGF) is the major cytokine growth factor which is overexpressed during cervical neovascularisation and is particularly increased during invasion and metastasis. It is 34 to 42kDa disulfide dimeric glycoprotein and modulates angiogenesis. In tumor growth or metastasis IL-6 and other cytokines act through the release of VEGF as demonstrated in several *in vitro* and *in vivo* studies. VEGF expression is increased both in squammous as well as adenocarcinoma of cervix and is related to lymph node metastasis and prognosis. P53 protein and the erb pathways are altered in response to the increased levels of local VEGF concentrations. As a result angiogenesis inhibiting therapies have been evaluated in advanced cervical cancer. Bevacizumab or VEGF receptor blocking analogue has been tried in combination with surgery and radiotherapy or chemotherapy. More than 450 cases with recurrent cancer have been followed up and have shown improved overall survival (Vici et al., 2014).

However Katanyoo et al. (2011) in a study in advanced cervical cancer cases did not observe any correlation between pretreatment circulatory levels of serum VEGF levels, median level, 611.3 pg/ml (range: 0.00-4,067pg/ml), and progression or with response to radiotherapy.

Interleukin-8

IL-8 or CXCL8 is a tiny molecule with a weight of 9 kDa and is secreted by macrophages and other immune cells like monocytes. T cells and NK cells in the body in response to inflammation. It is stored in the endothelial cells of the blood vessels. High tissue or circulating levels are associated with schizophrenia, obesity and certain conditions of inflammation like gingivitis and psoriasis. It has a predominantly chemotactic activity for several types of epithelial cells and also the endothelial cells hence it promotes neoangiogenesis or vascularisation. In carcinogenesis excess of IL-8 indicates growth and or metastasis of the tumor.

Wu et al. (2013) showed that patients of cervical cancer with whose cervical biopsy exhibited increased expression of IL-8 were likely to have lymph node metastasis. They further showed that cervical cancer cell lines also produced increased levels of IL-8, both in the cancer cells and in the supernatant fluid. In an "*in vivo*" model of athymic mice with HPV positive cervical cancer xenograft implant they demonstrated elevated levels of IL-8 in circulation and in cervical cancer tissue and these were associated with larger tumor size and metastasis. They further observed an abrogation in tumor size after blocking IL-8 *in vivo*.

Fugimoto et al. (2000) studied malignant tissue levels of cytokines IL-1α, IL-1β, TNF-α, IL-8, bFGF, VEGF, and PD-ECGF from 80 women treated surgically for invasive cervical cancer and followed them up for 24 months. They observed that 20 women with tissue IL-8 levels >1000pg/mg protein ended with extremely poor prognosis whereas remaining 60 women with <1000pg/mg protein of IL-8 had 67% survival rate. Tissue IL-6 levels also correlated with cancer tissue micro-vascular counts and Infiltrated Macrophage Counts. VEGF (Vascular Endothelial Growth Factor) tissue levels also correlated with microvascular counts thus indicating that IL-8 is the local angiogenic factor derived from infiltrating macrophages and it promotes local infiltration as well as metastasis. Plasma levels of IL-8 are also increased in elderly women with persistent HPV infection as compared to those who clear the infection indicating an early change and impaired immunity even before CIN or cervical cancer develops (Baker et al., 2011).

Interleukin-10

IL-10 is an anti-inflammatory cytokine with an 18.5 kDa mass and forms a homodimer with 37kDa molecular Mass. IL-10 shares homology with some viruses eg Epstein-Barr virus, cytomegalovirus. Under pathological circumstances it can lead to immunosuppression and improper clearance of viruses like HPV, HCV, HCV and other pathogens and persistence of these infections and tumours. It decreases TH1 types of cytokines (IL-12, IFN gamma) and increases TH2 types of cytokines (IL-4, IL-5, IL-13). IL-10 also decreases the proinflammatory cytokines IL-2, IL-6, IL-1β, IL-12, GM-CSF, TNF-α and IFN-γ (Asadullah et al., 2003; Bijjiga et al., 2013).

The sources of IL-10 have been identified as TH2 cells, monocytes, macrophages, B lymphocytes, eosinophils, mast cells and possibly keratinocytes. Multiple factors including cytokines, NFkB, and stress can lead to IL-10 release from different sites. IL-10 activity is mediated through IL-10 receptors which are situated mostly on immune cells. IL-10 may effect its actions by inhibiting NF-kB activity through dual mechanism; *i*) inhibition of IKK activity; *ii*) Blocking the binding of NFkB to DNA. IL-10 also inhibits VEGF and angiogenesis (Kohno et al., 2003; Lin et al., 2007).

Local levels of IL-10 in cervicovaginal secretions were increased in women with HIV-and HPV associated CIN (Mhatre et al., 2012). Serum levels of IL-10 were observed to be significantly higher in women with invasive cervical cancer and high grade CIN as compared to controls (Feng et al., 2012). They did not find any difference in serum levels of IL-10 in healthy women who were HR HPV positive or HR HPV negative indicating that defective immune reposes are more contributory to the carcinogenetic process rather than the infection per say.

IL-10 polymorphism has been reported and correlated

Table 1. *in vitro* Studies Related to Cytokines and Cervical Cancer Cell Lines

	Cytokine	Cell lines	Study	Reference
1	IL-1α, TNF- α	SiHa, CaSki, HT3, C-33A, C-41, ME-180, CXT-1, CXT-2, and CXT-3	Proinflammatory cytokines induce proliferation via an EGF dependent pathway.	Woodworth et al., 1995
2	IL-17	HeLa, ICI	Cell lines transfected with human IL-17 gene increased production of IL-6	Tartour et al., 1999
3	IL-10	K562, HPV E7-specific T cells, dendritic cells	IL-10 stimulates production of Th-1 cytokines and cytotoxicity of HPV specific CD8+ CTL	Santin et al., 2000
4	IL-6	SiHa, CaSki, HeLa, C33a	Inhibition of IL-6 receptors prevents MCP-1 production and thus help the tumor to escape the immune system	Hess et al., 2000
5	TGF-β1, IL-4,IL-12 IL-15 TNF-α, IL-10, IL-5, MCP-1	Cervical Cancer Cell Lines(CCCL), Normal Primary Epithelial Cell Lines (NPC)	Cervical cancer cells produce immunomodulatory cytokines and the pattern changes after malignant transformation	Hazelbag et al., 2001
6	IL-6	C33a	IL-6 prevents apoptosis of cancer cells via upregulation of Mcl-1	Wei et al. 2001.
7	CSF-1,c-fms, TGF-beta	HeLa, CaSki	Increased expression of CSF-1,c-fms, TGF-beta, Expression and cell motility blocked by blocking TGF-beta receptor	Kirma et al., 2007
8	IL-6, TGF-b, IL-10, VEGF. MCF	Human Cervical Cancer cells	Co-cultured with monocytes, M1 type changed to M2 type by Cancer cells	Heusinkveld et al., 2011
9	VEGF, TIMP1, MMP2, IL-6,IL-15	HeLa cells	Activation of Toll Like Receptors regulated the cytokine secretion in HeLa cell culture	Li et al., 2013
10	IL-8	SiHa, CaSki, HeLa	Suppression of IL–8 expression with shRNA and IL–8 Ab reduced cell proliferation and invasion	Wu et al. 2013

with some immune disorders and cervical cancer (Moore et al., 2010; Shekari et al., 2012). Interleukin -10 deficiency is observed in some diseases and a recombinant analogue (administered subcutaneously or intramuscularly) is undergoing clinical trial in rheumatoid arthritis, inflammatory bowel disease, psoriasis, organ transplantation, and chronic hepatitis C. The effect of IL-10 on tumours is variable and both inhibition or promotion of tumor growth have been reported (Asadullah et al., 2003). It can inhibit neovascularization or angiogenesis and metastasis. However IL-10 can directly stimulate tumor cells or inhibit immune system thus allowing tumor to escape the immune protection. IL-10 overexpression has been associated with tumor development.

IL-12

Interleukin-12 is a 74kDa heterodimer glycoprotein and is expressed by B cells, phagocytes and dendritic cells. IL-12 appears to be immunoprotective for cervical cancer. IL-1 and IL-6 induce IL-17/IL -23 pathway during cervical carcinogenesis. The reduced expression of the cytokine IL-12 in cervical biopsy specimens from invasive cancer cases was associated with a reduced immune response, and high IL-1 and IL-6 levels as seen by immunocytochemistry. IL-12p40 is a subcomponent of IL-12 and IL-23 and its mRNA expression was studied. Thus low IL-12 levels are associated with a very poor prognosis of cervical cancer. Chemokine CACL12/CAC×4-receptor was associated with pelvic lymph node metastasis (Murooka et al., 2005). Yang et al. (2007) observed positive lymph nodes when CACXR4 expression was positive in tissue samples. IL-12 has anti-neo-angiogenetic effect.

Adipokines

In view of the observed connection between adipokines and cancer Baker et al. (2011) have reported on plasma levels of adipokines resistin and sFas in older women (45 yrs+) with or without persistent HPV infection. Using multiplex cytokine assay they measures the following cytokines in plasma (Total N=100): Adipokines, adiponectin, resistin, tPAI-1, HGF, TNF-α, Leptin, and also IL-8, sVCAM-1, sICAM-1, sFas, and MIF. They observed a significant increase in plasma levels of resistin and sFas as also IL-8 and TNF-α in women with persistent HPV infection indicating impaired immune response.

in vitro Studies on Cytokines and Cervical Cancer

Considerable knowledge about cytokines and the mechanisms of action has been generated from *in vitro* studies on a variety of cervical cancer cell lines and recently normal epithelial cell lines as well as CIN (precancerous) cell lines also have become available. Some of these are summarized in Table 1.

in vivo studies on cytokines and cervical cancer

Various experimental studies have also been conducted to elucidate the role of various cytokines. Although difficult to establish the studies on chemically (eg. DES or) induced cervical cancer in immature rats and human cervical cancer xenograft. These are summarized in Table 2.

Based on these studies, we have shown the putative mechanisms of cytokine effects in cervical carcinogenesis through a schematic diagram (Figure 1).

Key- IL: Interleukin, TNF-α: Tumor necrosis factor α, TGF-β: Transforming growth factor-β, TNFR: Tumor necrosis factor receptor, TRAF: TNF receptor associated factor, JAK: Janus kinase, TYK: Tyrosine kinase, IKK: IκB kinase, PI3K: Phosphoinositide 3-kinase, Akt: protein kinase B, STAT: Signal Transducer and Activator of Transcription, NF-κB: Nuclear factor-κB, MMP: Matrix metalloproteinase, VEGF: Vascular

Table 2. *in vivo* Experimental Studies Related to Cervical Cancer and Carcinogenesis

|	Model	Study	Reference
1	Tumor bearing mice	Il-6 induction in tumor bearing mice by administration of various cytokines	Mackintosh et al., 1989
2	nu/nu mice	Mice transplanted with IL-17 transfected HeLa cells had larger tumors than control	Tartour et al., 1999
3	Rat model for CIN & Cancer cervix	CIN induced in young rats with Diethyl stibestrol (DES) and followed up with histology; serum IL-6 correlated with degree of CIN and invasion	Bustamam et al., 2008
4	Xenograft athymic mice model	Subcutaneous Xeno graft model of HPV positive SCC- IL8 expression increased, IL-8 blockade reduced tumor size	Wu et al., 2008
5	Nude mouse xenograft assay	IL-2 induced killer cells inhibited growth of KB-3-1 tumor growth in nude mouse xenograft depending upon the number of cells per mouse.	Kim et al., 2009
6	Nude mouse xenograft assay	Cisplatin resistant xenograft with or without IL-24 injections, IL-24 enhanced the anticancer effect of Cisplatin	Li et al., 2011
7	Nude mouse xenograft assay	Blockade of IL-8 with antibody in nude mice showed significant antitumor effect	Wu et al., 2013
8	Nude mouse model human cervical cancer	Serum cytokines IL-2, IL-4, IL-5, IL-10, GM-CSF, IL-6, IL-13, M-CSF and IL-18 were detectable but no difference in groups,	Scutter et al., 2013
	Xenograft Cidofovir resistant/nonresistant	Defective (IL-1, IL-6, IL-8, IL-9, IL-10) and interferon signalling pathways with drug resistance	
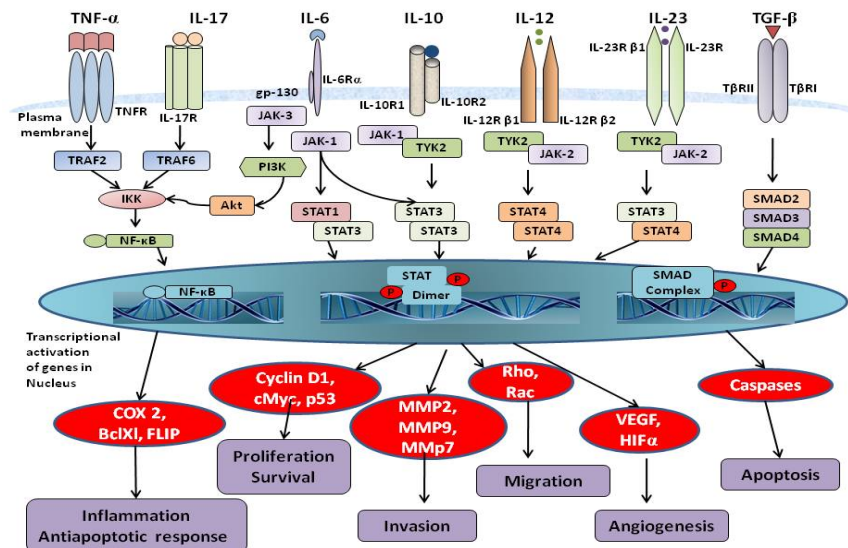
Figure 1. Schematic Diagram of Molecular Actions of Cytokines in Cervical Carcinogenesis

endothelial growth factor

Lastly several clinical studies have also been carried out and whilst some are briefly described below others are listed in Table 3.

Multiple cytokines

IL-17

Increased expression of this Interleukin is associated with cervical cancer cell growth along with increased evidence of IL-6 levels in tumor tissues. IL-17 may act through IL-6.

However, paradoxically, proapoptotic (TRAIL) and antiinflammatory (IL-10 and TGF-β) cytokines usually interfere with tumor development. IL-17 and IL-22 displayed a similar pattern of results, with higher serum level in LSIL patients, when compared HSIL patients (mean-pg/ml: 22.50 *vs* 12.20, and 168.2 *vs* 61.48) indicating compromised immunity in HSIL patients (Souza et al., 2013). IL-18 also known as IFN inducing factor (IGIF) or IL-1-gamma, induces IFN-g production from T lymphocytes and NK cells and acts synergistically with IL-12 to promote the Th1 response (Barksby et al., 2007).

Heusinkveld et al. (2011) studied the influence of human cervical cancer cells obtained from surgically treated cervical cancer patients on monocyte differentiation in tissue cell cultures and showed that the cancer cells either hampered monocyte to dendritic cell differentiation or skewed their differentiation toward M2-like macrophages. They showed that M2 differentiation was caused by tumor-produced PGE2 and IL-6. TGF-b, IL-10, VEGF. MCF was not important for this M2 differentiation.

Tjiong et al observed in an initial study that local levels of IL-6 in cervicovaginal fluid were high in cervical cancer cases. In a subsequent study they observed that whilst the levels of IFN-gamma did not differ in different groups, IL-12p40, IL-10, TGF-beta1, TNF-alpha and IL-1beta levels were significantly more in women with cervical cancer than in controls and in CIN (Tjiong et al., 1999; 2001).

Sharma et al. (2007) studied the production of cytokines from peripheral blood lymphocytes in 60 cases of cervical cancer, 35 cases of CIN (Cervical Intraepithelial Neoplasia) and 30 healthy women. They observed a decline in IL-2 levels in CIN III and cancer, and decline in IFN-gamma only in late stages of cervical cancer whilst an increase in the levels of IL-4 and IL-10 was found in CIN III and cancer cervix (p<0.001). They also observed a correlation with HPV 16/18 positivity. This indicates that there are constitutional disturbances in the immune system in precancerous stages as well as with advanced cancer.

Other newly detected biological molecules IL-19, IL-20, IL-22, IL-24 (mda-7), and IL-26 (AK 155) are IL-10 homologues and play an important role in immunoregulation

Table 3. Clinical Studies on Cytokine Levels in CIN and Cancer

No	Population	Samples/ end points	Cytokines	Remarks	Reference
1	Healthy women, CIN I,II,III, Cancer cervix	Peripheral blood lymphocyte culture	Soluble IL-2 receptor	Decreased in CIN III & Cancer	Hildesheim et al., 1997
2	Healthy women, HR-HPV positive cases, CIN, cancer cervix	Circulating levels	CSF-1	Increased in CIN III and cancer cervix and HR-HPV positive cases	Adam et al., 1999
3	CIN, Cervical cancer, Healthy controls	Cervicovaginal washings	IL-12p40, IL-10, TGF-beta1, TNF-alpha and IL-1beta	Increased in CIN and Cervical cancer	Tjiong et al., 2001
4	Cancer cervix, CIN, Healthy controls	Peripheral blood lymphocyte culture	IL-2,IFN-gamma,IL-4, IL-10	IL-2, IFN –gamma decreased and IL-4,IL-10 Increased	Sharma et al., 2007
5	Cancer cervix, healthy controls	Cervical biopsy tissues	CSF-1,c-fms, TGF-beta, TGBR	Increased expression in cancer tissue	Kirma et al., 2007
6	CIN, Cancer, Bacterial vaginitis, Control	Cervicovaginal Washings	IL-8, IL-10 and Nitrous oxide	Increased in CIN, Cancer	Tavares-Murta et al., 2008
7	Healthy controls, CIN, Cervical cancer	Plasma Concentrations	TNFalpha, TNFalpha receptors, TNFRI and TNFRII,IFNgamma, IL-2, IL-12, IL-4, and IL-10	IL-12, IL-2, IL-4, TNFalpha –no difference, Lower levels of TNFRII in CIN III, cancer IL-10 raised in CIN III, cancer	Bais et al., 2007
8	Cervical adenocarcinoma	Lymph node	CACX12/CACX4	Positivity associated with Lymph node metastasis	Yang et al., 2007
9	Women with persistent HPV vs women with clearance of HPV	24 cytokines in plasma and in PMBC supernatant	24 cytokines	IL1-alpha, IL-6,IL-8,TNF alpha,MIP,GM-CSF Increased in persistent HPV infection	Kemp et al., 2010
10	Persistent Low –Grade Intraepithelial Lesion (LSIL) in Pap smear	Pap smears, colposcopy and serum IL-6	Serum IL-6	significantly high in LSIL, Reduced with oral TO extract with regression in Pap smears	Joshi et al., 2011
11	Invasive cervical cases- biopsy proven, Followed up	Cervical biopsy from cancer cases	mRNA Expression of IL-12, IL-17, Il- 23, ICH- IL12, IL-6	IL-12 poor expression is associated with a very poor prognosis	Zijlmans et al., 2012
11	Women surgically treated for invasive cervical cancer	Cervical biopsy tissue concn of Interleukins	IL-1α, IL-1β, TNF-α, IL-8, bFGF, VEGF, and PD-ECGF	Very poor prognosis in women with >1000pg/mg IL-8	Fugimoto et al., 2013
12	Healthy women, CIN, Cervical cancer	Cervical biopsies	CXCL12,CXCR4, CXCL16,CXCR6	Increased in CIN, Cancer, Lymph node metastasis	Huang et al., 2013

of the lymphatic system (Asadullah et al., 2003). Their effects and mechanisms of actions are being studied.

Bais et al. (2007) studied the TH1 and Th2 cytokine response of Peripheral Monocyte Blood Lymphocyte (PMBL) cultures as well as from biopsies from women with precancer and cervical cancer and healthy controls. They observed that HRHPV positivity is associated with Th1 type of response and there is a change with progressive grades of CIN and cancer.

Kemp et al. (2010) conducted a longitudinal study in HPV positive cases and compared the circulating levels of 24 cytokines in 50 women who had persistent HPV infection (study group) *vs* 50 women in whom the HPV infection was cleared (controls) after a long term follow up for 5-6 years. Peripheral Monocyte Blood Culture (PMBC) was also studied and supernatant measured for cytokines. Plasma levels of IL-6, IL-8, TNF-α, MIP-1α, GM-CSF, and IL-1α were significantly higher in women with persistent HPV *vs* controls. The PBMC supernatant had higher concentrations of IL-6, TNF-α, and MIP-1 α in the study cases *vs* controls. The authors concluded that persistent HPV infection is associated with high levels of specified cytokines indicative of a deficient immune response.

Mbulaiteye et al. (2013) studied serum profile of 19 cytokines, including Th1-like cytokines, Th2-like cytokines, innate/inflammation cytokines, and cell development cytokines in 964 Nigerian women who were HPV positive. Significant changes were was restricted to 5 cytokines, TNF-α (Th1), IL-8 (Th2), eotaxin and MCP-1 (innate/inflammation), and G-CSF (cell development). They suggested that the abnormal cytokine patterns are related to cellular abnormalities which may arise from a defective immune response in some women, and not due to the mere presence of HPV infection.

Peghini et al. (2012) measured the levels of local cytokines in cervical biopsies from cases of invasive cancer, high grade CIN and low grade CIN *vs* healthy controls. High grade CIN was associated with increased Th1 cytokines whilst CIN 1 was associated with increased Th2 cytokines. Treg cytokine profile showed progressive increase with progressing disease indicating a poor cell mediated immunity with high grade lesions.

Lazarenko et al. (2014) have recently evaluated a panel of biomarkers for assessment of progression from healthy cervices to HPV infected, CIN and cancer. These included several cytokines (PBMC), HPV status, HSV antibodies, and newer biophysical techniques like cervical sonocervicography using ultrasonography which has shown a high specificity, sensitivity, positive predictive value and negative predictive value. They have proposed that the weak avidity or functional affinity of HSV antibodies also is an important factor in the weak immune response in CIN and cancer cases leading to poor TNF alpha levels and persistent HR HPV infection.

Cytokines as Markers of High Risk of Progression of CIN Grades

Many investigators have observed a strong correlation between cytokine expression in cervical biopsy specimens and the histologic grading. However biopsy is an invasive procedure and is particularly non-desirable in the younger women desirous of conserving menstrual and childbearing functions. Hence further studies were carried out by authors who have observed that the higher local or circulatory peripheral concentrations of some cytokines are indicative of a higher risk of progression from CIN 1 to CIN 2 or 3 and further to invasive cancer and metastasis (Adam et al., 1999; Fugimoto et al., 2000; Pardo-Govea et al., 2005; Bais et al, 2007; Sharma et al., 2007; Hong et al., 2010; Kemp et al., 2010; Ali et al., 2012; Mhatre et al., 2012; Peghini et al., 2012; Lazarenko et al., 2014). Souza et al. (2013) recently demonstrated higher serum concentrations of IL-17, mean-pg/ml: 22.50 in HSIL *vs* 12.20 in LSIL, and of IL-22, mean pg/ml 168.2 *vs* 61.48, p<0.05 respectively.

In another study plasma levels of cytokines SCF, GM-CSF, G-CSF, M-CSF and antigen SCC were determined in different groups of women in whom histological diagnosis of CIN or cancer was made after surgery. Many women with cancer or neoplasia had higher concentrations of these compounds than normal healthy women. M-CSF concentration was most consistently increased with increasing grades of neoplasia including cancer (Lawicki et al., 2012).

Din et al. (2014) observed the microRNA (miRNAs) arrays in cases with and without pelvic lymph node metastasis and noted that several of these with post-transcriptional target mRNA genes were altered in cases with metastasis. As many as 39 miRNAs were at least 4 timed altered in cases with metastasis, 22 being upregulated whilst 17 miRNAs were downregulated. Six of these targeted genes were associated with cell growth, differentiation, proliferation and migration.

Collection and standardization of concentrations in local cervicovaginal fluid is somewhat more tedious and expensive than measurement of circulating levels of cytokinre as these methods as immunoassays cane be easily standardized in majority for majority of cytokines. The more promising amongst them appear to be circulating levels of IL-6, IFN, IL-8, and IL-10.

HPV Dependent Cytokine Alterations

Alterations in some cytokines are specifically associated with HPV-related carcinogenesis whilst other cytokine alterations are independent of HPV Viral DNA. Bais et al. (2007) observed a significant difference in the cytokine response of Peripheral Monocyte Blood Culture (PMBC) from HR HPV positive *vs* HRHPV negative women. Further there were differences in cytokine concentrations due to the degree of cervical neoplasias. Specific HPV proteins like E2, E5, E6, and E7 have been found to be associated with certain cytokine dysfunctions. HPV 16 E5 and E7 oncoproteins promote the regulatory element of TGF-β. E2, E6, E7 specific T- cell responses have been determined in HPV positive women in long term follow ups for intraepithelial neoplasia. These modulate and favour the immune escape phenomenon during transformation of cancer cells. HPV E2 protein induces the IL-10 mRNA activity in HPV infected epithelial cells. IL-10 causes immune suppression and allows viral persistence and allows survival of transformed cells (Bhairavabhotla, 2007; Bermudez-Morales, 2008; 2011). Baker et al. (2011) observed that circulating levels of the adipokine, resistin, IL-8 and TNF-α were higher in older women with persistent HPV infection. They also observed higher IL-6, TNF-α and IL-12 production from PMBC *in vitro* after addition of resistin, thus indicating a correlation between, obesity associated cytokines, persistent HPV infection, and cervical neoplasia.

Immunotherapy with Cytokines/Blocking Antibodies

Chemotherapy has not been very successful in cervical cancer, both because the cancer is not responsive to the drugs, and also because most of these drugs also attack normal tissues all over the body leading to intolerable side effects. Targeted therapy or treatment directed towards specific cytokines or their receptors is less likely to affect normal tissue and hence is currently being evaluated for several cancers including cervical cancer. Soon after the observation of IFN-α and IL-2 deficiency in cervical cancer and intraepithelial neoplasia the products were developed in an injectable form for use in advanced cancer. Systemic intravenous, subcutaneous or intramuscular injections or local intralesional cervical tissue injections have been studied with follow up studies which show disappearance of local lesions in some cases. In some cases the systemic side effects of the biological have been considerable and there is still a lot of scope for development in this area. Ramos et al. (2010) observed that women with a satisfactory response to local injections of IFN-α2 had increased respose of cytokines IFN-α, TNF-α, and IL-2 whilst therapeutic failures were associated with increased levels of IL-4 and TGF-β. HPV viral load was also reduced in responders. In another study involving serum measurement of cytokines IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, TNF-α, TGF β in cases of CIN 2 treated with intralesional injections of IFN-α 2b, the serum level of IL-12 was increased in responders (Misson et al., 2011). New biological treatment avenues are being explored with several cytokines, analogues or with antibodies against them depending on their mechanism of action and these have been recently reviewed. IGF-1 R directed antibodies have been studied in preclinical and clinical trials and have shown promise. Maximum patients were enrolled (N=450) with Bevacizumab targeting VEGF. Trials with combination therapies may offer a longer life with better quality of life.

The serine-threonine kinase (mTOR) which regulates cell cycle is aberrant in cervical cancer and activates IGFR and EGFR. HPV oncoproteins interact with this pathway hense temsirolimus which inhibits the pathway is currently being evaluated in Phase 3 trial with or without radiotherapy or chemotherapy. A Phase 2 trial is ongoing

with Brivanib monotherapy directed towards VEGF and FGFR. A progression free survival (PFS) of at least 6 months is one of the end points along with a tolerable incidence of side effects.

It is important to note that immunotherapy may be associated with several minor or major side effects which may be constitutional (fever, fatigue, arthralgia, headache, malaise, vomiting etc), heamatologic (bone marrow suppression), cardiovascular, renal, gastrointestinal, neurologic and muscular, pulmonary side effects. Although with the prolongation of life, Progression Free Survival (PFS) and Overall Survival (OS) are marginally improved, the quality of life may be adversely affected and there is scope for developing safer and tolerable alternatives (Liu et al., 2004; Moschos et al., 2007; Bruchim et al., 2013; Vici et al., 2014).

Conclusions

Cervical cancer is usually preceded by persistent High Risk HPV infection which develops into Low Grade-or High Grade Intraepithelial Lesions (LSIL,HSIL) detected in Pap smear or as Cervical Intraepithelial Neoplasias (CIN Grade 1,2,or3) in cervical biopsy. Colposcopy and ultrasonography have added to the diagnostic accuracy of precancer or CIL. The carcinogenesis occurs over several years and involves multiple changes/insults to the DNA (Weinberg, 2007). Not all cases with LSIL or HSIL will develop cancer (Moscicki et al., 2004; Weinberg, 2007; Melnikow et al., 2009) but there is ample time to detect and treat precancerous conditions to prevent cervical cancer. It is very important to identify precancer cases with a higher risk of cancer development so that they can be followed up frequently and treated more aggressively if cervical cancer is to be prevented. In this article we have reviewed the possible role of cytokines in cervical carcinogenesis and in prediction as well as management of CIN and advanced cancer. Genetic susceptibility is also noted eg P53, IL-1 beta, IL-6 gene, IL-6 receptor mutations, TNF-alpha, IL-17, IL-8. It is equally important to give weightage to other high risk factors like i) High Risk (HR) HPV positivity; ii) Early age at first coitus; iii) Multiparity; iv) Multiple sexual partners; v) Smoking or Tobacco intake in any form; vi) Associated Sexually Transmitted Diseases (STDs), in particular, HPV, HSV, HIV, Chlamydiasis, Bacterial vaginitis, particularly when persistent or chronic. However it is beyond the scope of this article to discuss these factors. General susceptibility in the form of nutritional deficiencies, particularly of i) Vitamin A; ii) Vitamin B complex; iii) Folate; iv) Zinc; v) Selenium have been associated with cervical precancer and cancer.

A delicate balance exists between proinflammatory and anti-inflammatory cytokines and their disturbances are observed in many acute conditions like pyrexia, rheumatic disorders and cancers. Since all cytokines are physiologically essential mere presence of a cytokine is not diagnostic or prognostic, and sometimes the increases are related to the body's defense mechanisms. However, abnormal increases or decreases due to carcinogens or due to genetic variations like IL-6, IL-8 or IL-10

polymorphism can increase or decrease the risk of cervical cancer. Circulating levels of cytokines IL-2, IFN-a β, IL-2, IL-8, IL10 can be useful in identifying women at higher risk of developing cervical invasive cancer when detected with LSIL or HSIL, and risk of metastasis when detected with cancer. Circulating or tissue levels of IL-6, IL-8 and IL-10 can be of additional value in the prognosis of patients with advanced stage disease and may help the decision making processes in favour of or against major surgery, selection of chemotherapy, monitoring of chemoprevention trials, effects of HPV immunization, effects of treatment regimens for CIN, irradiation or combination therapies including immunotherapy either in CIN or in advanced disease.

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