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Abstract.

Background: The Nuclear Factor kappaB (NF-kB) family consists of transcription factors that play a complex and essential role in the regulation of immune responses and inflammation. NF-kB has recently generated considerable interest as it has been implicated in human cancer initiation, progression and resistance to treatment. In the present comprehensive review the different aspects of NF-kB signaling in the carcinogenesis of cancer of the uterine cervix are discussed. NF-kB functions as part of a network, which determines the pattern of its effects on the expression of several other genes (such as crosstalks with reactive oxygen species, p53, STAT3 and miRNAS) and thus its function. Activation of NF-kB triggered by a HPV infection is playing an important role in the innate and adaptive immune response of the host. The virus induces down regulation of NF-kB to liquidate the inhibitory activity for its replication triggered by the immune system leading a status of persistant HPV infection. During the progression to high grade intraepithelial neoplasia and cervical cancer NF-KB becomes constitutionally activated again. Mutations in NF-kB genes are rare in solid tumors but mutations of upstream signaling molecules such as RAS, EGFR, PGF, HER2 have been implicated in elevated NF-kB signaling. NF-kB can stimulate transcription of proliferation regulating genes (eg. cyclin D1 and c-myc), genes involved in metastasis, VEGF dependent angiogenesis and cell immortality by telomerase. NF-kB activation can also induce the expression of activation-induced cytodine deaminase (AID) and the APOBEC proteins, providing a mechanistic link between the NF-kB pathway and mutagenic characteristic of cervical cancer. Inhibition of NF-kB has the potential to be used to reverse resistance to radiotherapy and systemic anti-cancer medication, but currently no clinicaly active NF-kB targeting strategies are available.

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Introduction

Cervical cancer is the second most prevalent cancer seen in woman worldwide, with about 500.000 cases and over 270.000 deaths estimated annually (Chauhan et al., 2009). Over the last decades only limited progress has been made in the systemic treatment of patients with advanced or recurrent cervical cancer. The etiological role of infection with high-risk papilloma viruses in cervical carcinoma is well established (zur Hausen, 2009). Somatic mutations in PIK3CA, PTEN, TP53,STK11, KRAS, MAPK1, HLA-B, EP300, FBXW7, NFE2L2, TP53, ERBB2, as well as several copy number alterations have been implicated in the pathogenesis of cervical carcinomas (Ojesina et al., 2014, Xiang et al., 2015). In a recent study we used an in silico approach to search for potential driver pathways of cervical carcinogenesis and candidate targets for treatment (van Dam et al., 2016). A PPI-network consisting of 5 signaling modules was identified. These were associated with MYC signaling, cell cycle deregulation, TGF-β and NF-kB signaling, MAPK signaling and chromatin modeling. Disruption of these genetic networks by HPV infections has been demonstrated in vitro and in humans (Altomare et al., 2013). In the present comprehensive review of the literature the NF-kB signaling network in cervical cancer will be discussed in detail.

The NF-kB family

The transcription factor NF-kB was discovered in 1986 as a nuclear factor that binds the enhancer element of the immunoglobulin kappa light-chain of activated B cells (therefor called after the abbreviation: NF-kB) (Sen and Baltimore, 1986). The NF-kB family consists of transcription factors that play a complex and essential role in innate immunity, inflammation, viral replication and the initiation and progression of cancer. Five members have been identified in mammals; p65 (RelA), RelB, c-Rel, NF-kB1 (p105/p50) and NF-kB2 (p100/p52) (Baldwin, 2001, Barnes and Karin, 1997, Caamano and Hunter, 2002, Hoesel and Schmid, 2013, Perkins, 1997, Perkins, 2004). In contrast to the other family members, NF-KB1 and NFKB2 are synthetized as pro-forms (p105 and p100) and then proteolytically processed to p50 and p52 (Hoesel and Schmid, 2013). All five members of this protein family can form a variety of homo- or heterodimers undergoing phosphorylation and other posttranslational modifications, which are essential for their activation, crosstalk and translocation to the nucleus. They can bind target DNA and induce different target genes (Wong et al., 2011). The classic form of NF-kB is a heterodimer between p65 (RelA) and p50 subunits. In most quiescent cells these dimers are bound to inhibitory molecules of the IkB (inhibitors of NF-kB) family of proteins (Hoesel and Schmid, 2013). IkB proteins are characterized by ankyrin repeats, which associate with the DNA binding domains of the transcription factors making them transcriptionally inactive. P105 and p100 (the precursors of p50 and p52, contain similar ankyrin repeats which are cleaved upon maturation and can as such function as their own internal inhibitors (Hoesel and Schmid, 2013). Dimers of p50 and p52 not containing a transactivation domain that can bind to the NF-kB elements of gene promoters, act as transcriptional repressors (Hayden, 2012). However, when p50 or p52 are bound to a family member having a transactivation domain, such as p65 or RelB, they act as a transcriptional activator. The variations of contexts in which dimers are formed are enormous (Campbell and Perkins, 2004, Oeckinghaus and Ghosh, 2009).

Activation of NF-kB can occur within minutes by release from IkB or by cleavage of the inhibitory ankyrin repeat domains of p100 and p105 (Hoesel and Schmid, 2013, Tan et al., 1999, Wu et al., 2013, Zandi et al., 1997). This process is catalyzed by an enzyme complex containing IkB kinases (IKK1 and IKK2) and at least one non-catalytic accessory protein (NF-kB Essential Modulator: NEMO) (Klement et al., 1996, Oeckinghaus and Ghosh, 2009, Pahl, 1999). A great variety of stimuli can trigger this type of NF-kB activation (eg microbial products and pro-inflammatory cytokines such as TNFalpha and IL-1) and it is mediated through Toll-like receptors (TLRs), tumor necrosis factor receptors (TNFR), antigen receptors and Interleukine-1 receptors (IL-1R). (Perkins and Gilmore, 2006, Oeckinghaus and Ghosh, 2009). This so-called canonical pathway is generally anti-apoptotic, tumor promoting and has also been implicated in the pathogenesis of chronic inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease and asthma (Figure 1) (Coward et al., 2004). An alternative pathway of NF-kB activation, the non-canonical or atypical pathway, is independent of the activity of IKK2 and NEMO and has different functions, sometimes tumor

suppressing and facilitating apoptosis (Figure 2) (Bentires, 2001). Important receptors activating the non-canonical pathway are B-cell activation factor (BAFFR), CD40 and Receptor activator of NF-kB ligand (RANKL) (Bonizzi et al., 2004). This leads to activation of the NF-kB inducing kinase (NIK), which phosphorylates and activates predominantly IKK1 resulting in ubiquitination and partial degradation of p100 to p52 (Xiao et al., 2001). Activation non-canonical NF-kB pathway results in several mixes of subunits targeting different promoters and enhancers through differences in DNA-binding affinity. (Hoesel and Schmid, 2013, Perkins and Gilmore, 2006, Lawrence, 2009, Perkins, 2004, Baldwin, 2001).

Studies show that the activation of NF-kB can have contrasting roles in same-cell lineage, depending on physiological context (Campbell and Perkins, 2004, Lawrence, 2009, Natoli et al., 2005). This can be explained by the fact that NF-kB does not function alone but is part of a network, which determines the pattern of its effects on the expression of several other genes (such as crosstalks with upstream kinases, chromatin modifiers, reactive oxygen species, p53, STAT3 and miRNAS) and thus its function (Hoesel and Schmid, 2013) (Figure 3 and 4). This complexity has lead to many apparent contradictions in literature.

The NF-kB signaling pathway in inflammation

Based on studies which showed that activation of the canonical NF-kB signaling pathway can induce the expression of inflammatory cytokines, chemokines and their receptors, NF-kB has long been considered to be a prototypical pro-inflammatory signaling pathway stimulating the immune system as a response to physical, physiological and/or oxidative stress. NK-kB activity can on the other hand also be elevated by inflammatory cytokines, creating the possibility for a positive feedback mechanism (Pahl, 1999, Perkins, 2004). In tumors with high NF-kB activity, the accumulation of proinflammatory cytokines directly contributes to the protumorigenic microenvironment, which is critical for tumor initiation and progression (Xia et al., 2014). NF-kB has the ability to activate adhesion molecules and chemoattractant proteins essential for the recruitment of inflammatory cells, and to combat infections such as HPV (Disis, 2010). Moderately elevated levels of NF-kB activity are often seen in chronic inflammatory conditions. The fact that constitutive activation of NF-kB exerts a pro-tumoregenic effect is illustrated by the observation that patients with chronic inflammatory diseases have a higher risk to develop cancer, similar to immunosuppressed patients (Hoesel and Schmid, 2013). On the other hand the NF-kB protein p50 has been shown to favor immunosuppression by suppressing M1-polarization and inducing M2-polarization of macrophages (Porta et al., 2009). In some studies NF-kB also had a (contradictory) role in the expression of anti-inflammatory genes, for example causing the induction of leucocyte apoptosis during the resolution of inflammation. In fact inhibition of NF-kB can sometimes cause a prolonged inflammatory response. Therefor one can conclude that both pro- and anti-inflammatory NF-kB related mediators are produced during inflammation, depending on time and tissue. The balance between these factors seems to dictate disease progression (Baldwin, 2001, Greten et al., 2007, Lawrence, 2009, Lawrence et al., 2001).

NF-kB as tumor promoter

Both the classical and alternative NF-kB pathways (to various extents) are activated in many types of cancer and this is often associated with a bad prognosis (Hoesel and Schmid, 2013). Activating mutations in NF-kB genes were first found in B-cell lymphoid malignancies but are rare in solid tumors (Xia et al., 2014). However, it has been shown that mutations of upstream signaling molecules (such as RAS, EGFR, PGF, HER2) often lead to constitutive activation of NF-kB in solid malignancies (Chaturvedi et al., 2011). In addition NF-kB is activated by continuous release of cytokines by macrophages in the tumor environment (Hoesel and Schmid, 2013). The effect of these signaling molecules and cytokines on signalling in the NF-kB pathway strictly depends on the cell type or the microenvironment. The human immune system can play a dual role being either tumor promoting creating more aggressive tumors, or can be host protective. This is called cancer immunoediting (Disis, 2010, Dunn et al., 2004, Hoesel and Schmid, 2013, Smyth et al., 2006). NF-kB activation can increase cell survival, inhibiting programmed cell death by stimulating the transcription of anti-apoptotic genes (Kucharczak et al., 2003). This provides mechanisms to withstand the physiological stress during inflammation, thus playing an important role as co-factor in the initiation of tumors (Hoesel and Schmid, 2013, Perkins and Gilmore, 2006). NF-kB can also stimulate transcription of proliferation regulating genes (eg. cyclin D1 and C-MYC (Guttridge et al., 1999, La Rosa et al., 1994, Perkins, 1997), genes involved in metastasis (eg. cellular adhesion molecules and matrix

metalloproteinases), VEGF dependent angiogenesis (Xie et al., 2010) and cell immortality by telomerase (Table 1). NF-kB seems to form the critical link between chronic inflammation and cancer. The microenvironment of a solid tumor contains high levels of inflammatory cytokines and hypoxic conditions, both stimulating activation and nuclear translocation of NFkB (Karin et al., 2002, Perkins, 2004). NF-kB activation can also induce the expression of activation-induced cytodine deaminase (AID) and the APOBEC proteins. The AID/APOBEC family is an important contributer to cancer development and clonal evolution of cancer by inducing collateral genomic damage due to their DNA deaminating activity (Rebhandl et al., 2015). It has been shown that the APOBECs can induce mutations in p53, c-MYC and other genes, which are crucial players in the development of cervical cancer (Matsumoto et al., 2007).

Active STAT3 is responsible for a number of genes that promote cell proliferation and or prevent /cell death in cervical lesions such as Cyclin D1, C-MYC, BCL-xl, survivin, VEGF, and MCL-l (Aggarwal et al., 2009). STAT3 and NF-kB work together in a network. They regulate a set of genes encoding chemokines and cytokines and control a number of target genes including cell cycle control and anti-apoptotic genes. P65 and p50 NF-kB interact physically with STAT3, facilitating STAT3 recruitment to NF-kB and vice versa. By the recruitment of acetyltransferase p300, STAT3 can modify p65 (RelA) post-translationally. Acetylation of NFkB causes a prolongation of its nuclear retention. This affects NF-K activity with chronic stimulation causing the release of cytokines like IL6, which activates STAT3. Thus activation of STAT3 causes prolonged activity of NF-kB, causing a positive feedback mechanism (Dauer et al., 2005, Grivennikov and Karin, 2010, Lee et al., 2001, Yang et al., 2007).

NF-kB as tumor suppressor

There is growing evidence that in some circumstances NF-kB can function as a tumor suppressor, inhibiting tumor growth and promoting apoptosis. Full activation of NF-kB is accompanied by high activity of cytotoxic immune cells against cancer cells in early stages, which is called tumor immunosurveillance (Disis, 2010, Hoesel and Schmid, 2013). It has been hypothesized that in the early stages of cancer NF-kB can inhibit tumor growth, but the accumulation of mutations may lead to a loss of tumor suppressor functions and the oncogenic features of NF-kB can become more dominant. This two-step mechanism is probably tumor- and cell-type specific. Tumor suppressors can recruit the NF-kB subunits to their pathways inhibiting cancer development by converting them from activators to repressors of tumor promoting genes. NF-kB can stimulate the expression of apoptosis-inducing genes such as death receptors 4 and 5 and Fas (Perkins, 2004, Ryan et al., 2000). P53 induces cell-cycle arrest or cell death and can be activated by DNA damage, oncogene activation and cellular stress (Sionov and Haupt, 1999). Some studies suggest that NF-kB subunits (RelA) are essential to be recruited to the p53 tumor suppressor pathway to enhance p53-induced cell death. Under these circumstances NF-kB becomes a facilitator of apoptosis. P53 can also regulate the activity of p52 subunit of NF-kB. Therefor it maybe theoretically counterproductive to inhibit NF-kB in tumors that retain the wild type p53 (Ryan et al., 2000). However, another study showed that NF-kB contribution is more complex, cooperating with or antagonizing p53 (Tergaonkar and Perkins, 2007). Up-regulation of anti-apoptotic genes can counteract the function of p53 as a tumor suppressor. Activation of the non-canonical NF-kB pathway by IKK-alpha is an important regulator of tumorgenesis through inhibition of p53 activity (Tergaonkar and Perkins, 2007). It is clear that the interplay between NF-kB and p53 needs more study (Barkett and Gilmore, 1999, Rocha et al., 2003, Ryan et al., 2000, Tergaonkar and Perkins, 2007). Maybe the best way to look at NF-kB is as a stress response factor. The stimulus and the cell type involved may determine whether NF-kB leads to cell death or survival (Baldwin, 2001).

The Human Papillomavirus and NF-kB

Undifferentiated proliferating keratinocytes, presumably stem cells, are the initial target for HPV infections (Kadaja et al., 2009). Papillomaviruses have developed mechanisms to adapt to the normal cellular growth control pathways and to adjust their DNA replication and maintenance cycle to contend with the cellular differentiation. After successful infection of a keratinocyte, the virus expresses E1 and E2 proteins, which are necessary for replicating and maintaining the viral DNA as a circular episome (Kadaja et al., 2009). Nakahara et al showed that HPV E1 induces NF-kB activation thereby limiting E1 dependent genome replication of HPV16 (Nakahara et al., 2015). This implicates that NF-kB mediates a negative feedback loop to regulate HPV replication and that this feedback loop

could be associated with the control of the viral copy numbers. HPV DNA integration is a very early event in cervical carcinogenesis resulting in a situation where the mixed (episomal and integrated) pattern becomes the most prevalent state. Loss of HPV E2 during HPV integration into the host genome results in the constitutive activation of the viral oncogenes E6 and E7 (Table 2). E2 inhibits endogenous E6 gene expression and sensitizes cervical cancer SiHa cells to TNF-alpha induced NK-kB activation concurrently increasing senescence and survival. This indicates a dichotomous role for E2 as an oncogene and a tumor suppressor in HPV induced carcinogenesis (Prabhavathy et al., 2015).

E6 and E7 use multiple mechanisms to evade host immune surveillance (allowing viral persistence), and to deregulate the cell cycle and apoptosis control, thus facilitating the accumulation of DNA damage and cellular transformation. The best characterised activity of HPV16 E6 is its ability to degrade the tumour suppressor protein p53 via the proteasome pathway. HPV16 E7 protein binds the hypo-phosphorylated form of pRb, promoting its degradation via the ubiquitin-proteasome pathway and the progression of the cells into S phase (Ghittoni et al., 2015). The expression of the fully functional viral oncoproteins E6 and E7 is necessary for the maintenance of the extrachromosomal forms of HPV DNA, as they create a cellular environment to allow HPV maintenance and abrogate the checkpoints that block the long-term retention of extrachromosomal DNAs (Garner-Hamrick et al., 2004).

NF-kB is playing an important role in the innate and adaptive immune response of the host. The HPV-16 E6 and E7 proteins regulate NF-kB expression, but there are conflicting data whether they stimulate (James et al., 2006, Nees et al., 2001) or suppress NF-kB activation (Havard et al., 2005, Spitkovsky et al., 2002). Growth rate, cell type and context of the signals seem to be important determinants. The balance between the different types of dimerization of NF-kB family members is a crucial factor. P50 and p52 do not contain a transactivation domain, and as a consequence dimers of p50 and/or p52 binding gene promoters act as transcriptional repressors. The dimerization of RelB/RelA (p65) can also form a transcriptionally inactive complex (Hayden and Ghosh, 2012, Marienfeld et al., 2003). Nees *et al.* showed that HPV E6 increased the expression of functional components of the NF-kB signal pathways and enhanced the NF-kB DNA binding activity, which was associated with an increase in pro-inflammatory cytokines. The co-expression of E6 and E7 proved to be even more efficient in this context (Nees et al., 2001). This was shown in human ectocervical keratinocytes, but not in cells from the transformation zone (TZ), which is the critical zone for the progression on premalignant cervical lesions into cervical cancer (Burghardt and Ostor, 1983). HPV16 long control region (LCR) has a functional NF-kB binding site for NF-kB to act as a repressor of the HPV transcription. The virus needs a mechanism to liquidate this inhibitory activity for its replication. HR-HPV E7 can accomplish this by attenuating the IKK complex, preventing NF-kB nuclear translocation and its binding to DNA elements such as the LCR of HR-HPV (Fontaine et al., 2000, Nees et al., 2001, Spitkovsky et al., 2002). HR-HPV E6 seems to inhibit the transcriptional activity of NF-kB (p65) within the nucleus (Patel et al., 1999).

A recent study of Vandermark *et al. (Vandermark et al., 2012)* showed that the NF-kB activity was significantly higher in early passage of HPV, but immortalization decreased NF-kB activity and expression of its responsive genes in cells of the transformation zone. Inhibition of NF-kB by an IkB repressor mutant increases colony formation and immortalization by HPV16. HPV16 E6/E7 proteins inhibited basal and TNF-alpha-inducible NF-KB activity in human epithelial cells cultured from the transformation zone. Activation of NF-kB by constitutive expression of p65 inhibits proliferation and immortalization. It therefor seems likely that at the initial phase of HPV infection NF-kB activity is triggered as a part of the normal host innate immune response, but that later on down regulation of NF-kB becomes a mechanism of HPV to promote a persistent infection. HPV-16 also interferes with specific NF-kB related pathways including a decrease in expression of major histocompatibility genes (Georgopoulos et al., 2000), interferons, β-defensines and cytokines (Diamond et al., 2008, Hayden et al., 2006, Nees et al., 2001). It abolishes the Toll-like receptor-9, a mechanism to suppress the host immune response (Nees et al., 2001). This may lead to immortalization of the cells (Diamond et al., 2008). Activation of NF-kB results is crucial changes in the tumor microenvironment favoring carcinogenesis (Pikarsky et al., 2004). This is illustrated by an interesting case control study by Pallavi et al (Pallavi et al., 2015). These authors noticed that HPV–infected postmenopausal women carrying insertion allele NF-Kb1-94 polymorphism in association of a GG genotype of NF-KbIa 3'-UTR polymorphism were more suspectible to develop cervical carcinoma. One can conclude that NF-kB acts as a tumor suppressor in the initial stages of HPV infection cervical cancer cells, but seems to be

down regulated of during cancer initiation. The mechanisms by which NF-kB inhibits cell growth and immortalization of normal cervical cells is incompletely understood and needs further study.

NF-kB and cervical cancer progression

Only a minority of patients infected with HPV develop a persistent cervical inflammation and eventually a precursor lesion and invasive cervical cancer. As the latency time for these events is very long it is likely that coincidental events (ie unavoidable errors associated with DNA replication) switch the balance from chronic HPV inflammation into a cancer (Tomasetti et al., 2017). APOBEC enzymes are important players in the defense against viral infections and their involvement may be important in the early steps of carcinogenesis of cervical cancer. One APOBEC family member, the activation induced deaminase (AID), is expressed in B lymphocytes and participates in the process of hypermutation and class switch recombination to antibody generation (Pham et al., 2005). APOBEC3G is involved in the response to retroviruses acting on viral cDNA to elicit mutagenesis in the viral genome (Henderson et al., 2014). Halemano et al (Halemano et al., 2014) showed that mouse APOBEC3 mutates immunoglobulin heavy variable genes during retroviral infections, and thus generates virus-specific neutralizing antibodies. Maruyama et (2016) demonstrated that the classical NF-kB pathway plays an important role in the transcriptional regulation of APOBEC3B in various cancer cell types (Maruyama et al., 2016). Leonard et al (Leonard et al., 2015) could show that the protein kinase C (PKC)/NF-κB pathway specifically induces APOBEC3B. PKC activation by the recruitment of RELB (but not RELA) to the *APOBEC3B* promoter, also implicating noncanonical NF-κB signaling. Henderson et al (2014) provided evidence that APOBEC activity is a key driver of PI3K mutagenesis and HPV induced transformation.

It has been mentioned earlier that functional mutations in NF-kB signaling pathway are rare events in solid tumors. Several studies have indicated that NF-kB is constitutively activated during cervical cancer progression (RM et al., 2016). A linear relationship was seen between the increasing grade of cervical carcinoma in situ and the intensity of cytoplasmic NF-kB expression. This suggests a tumor-promoting role for NF-kB during the progression of cervical cancer. In normal cervical tissue and low-grade squamous intraepithelial lesions (LSIL) p50, p65 (RelA) and IkB-alpha are localised in the cytosol, in high-grade SIL and squamous cell carcinoma (SCC) NF-kB translocates to the nucleus. This upregulation occurs relatively late in carcinogenesis. Nair *et al.* showed a decrease of IkB-alpha in the cytoplasm was seen during cancer progression, suggesting IkB-alpha undergoes degradation in the advanced stages of cervical cancer. There was a trend of gradually increased nuclear NF-kB expression and DNA binding from normal to precancerous (squamous intraepithelial lesions) and carcinoma tissue, associated with disease development, but no HPV data were provided in this study.

Prusty et al also observed a gradual increase in binding activity and expression of NF-kB from LSIL to SCC. These authors showed that the p50 subunit which generally heterodimerizes with p65 for its transcription factor appears to form a p50/p50 homodimer instead of the classic p50/p65 heterodimer, thus inhibiting transcriptional activity due to the lack of a transactivation domain (Bohuslav et al., 1998, Prusty et al., 2005). There was a gradual increase in the expression and nuclear localization of p50 and a parallel decrease of p65 expression as the lesions progressed. Nuclear translocation and localization of p65 was found, but not involved in a dimer formation (Prusty et al., 2005). Other studies did not find a significant difference in activity of p52, c-Rel en RelB (Branca et al., 2006, Li et al., 2009, Nair et al., 2003, Prusty et al., 2005). Branca *et al.* showed in an immunohistochemical study in a series of 150 squamous carcinomas of the uterine cervix and 152 CIN lesions that overexpression of nuclear NF-kB is significantly associated with the progression of CIN3 to cancer (OR 21.9; 95% CI 2.96-161; p=0.0001), but that cytoplasmatic NF-kB showed only a small increase in expression (OR: 3.55; 95% CI 1.79-7.05) (Branca et al., 2006). Their hypothesis is that this can be explained by a mechanism in which HR-HPV escapes from the transcriptional control of NF-kB, ie E7 mediated impaired nuclear translocation of cytoplasmatic NF-kB and E6 conditioned attenuated NF-kappaB (p65) dependent transcriptional activity as discussed above. Cytoplasmic NF-kB expression remains detectable throughout the progression in to cervical cancer. Some IkBalpha phosphorylation takes place in progression CIN lesions (Nair et al., 2003), explaining the modest shift from cytoplasm to the nucleus in high-grade lesions. Neither cytoplasmic nor nuclear NF-kB expression predicts clearance or persistence of HR-HPV (necessary for cervical cancer progression) after treatment, and this had no prognostic value. A positive correlation between NF-kB expression and more aggressive behavior

of invasive cervical cancer was not found (Branca et al., 2006). Recently is was suggested that the interplay between NF-kB and cystic fibrosis transmembrane conductance regulator (CFTR) can be involved in the development of cervix cancer. Studies show that they are co-expressed in cervical cancer (Li et al., 2009, Nair et al., 2003, Peng et al., 2012), NF-kB mediating the expression of CFTR. Wu Zu *et al.* showed that from normal tissue to cancer specimen's expression level of CFTR and NF-kB increased progressively. Co-expression was a significant independent prognostic factor for cervical cancer and higher expression level of CFTR and NF-kB was significantly associated with poor prognosis: advanced FIGO stage, poorer histological grade, lymph node metastasis, worse survival rate and deeper invasive growth. Validation of these findings in a larger cohort is recommended. The synergistic (or agonistic) effects of CFTR and NF-kB needs further investigation in malignancies (Wu et al., 2013). Some studies suggest that CFTR is a suppressor of NF-kB - mediated inflammatory signaling (Hunter et al., 2010, Vij et al., 2009). The negative impact of CFTR on NFkB mediated innate immunity may be responsible for more aggressive behaviour of cervical cancer.

Functional links between NF-KB and other transcription factors including STAT3, SMAD 3 and 4, are numerous (Hoesel and Schmid, 2013). NK-kB and STAT3 cooporatively regulate a number of target genes including antiapoptotic and cell cycle control genes, and genes encoding for cytokines and chemokines (Yang et al., 2007). STAT3 regulates expression and function of p53 by binding its promoter region resulting in a decrease of de novo expression. It influences p53 response genes and thus prevents p53-mediated tumor cell apoptosis. P53 prevents phosphorylation of STAT3, so they antagonize expression of each other. By reducing p53 tumor cells allow STAT3 mediated growth and survival without mutation in the early stage. Blocking STAT3 induces the p53-mediated tumor cell apoptosis and facilitates the inhibition of tumor cell growth, showing possibilities for STAT3 as therapeutic target (Niu et al., 2005).

Cyclooxygenase-2 (COX-2) has a role in cervical cancer progression by increasing lymph node metastasis and resistance to radiotherapy-induced apoptosis. (Kim et al., 2003) (Kim et al., 2009) (Ishikawa et al., 2006) (Kulkarni et al., 2001) NF-kB plays an important role in the activation of COX-2 in cancer cells. (Surh et al., 2001) HPV E5 activates epidermal growth factor receptor (EGFR), but does not inactivate tumor suppressor proteins like E6 and E7. (Crusius et al., 1998) The COX-2 promoter region has three important binding sites: NF-kB, NF-IL6 and cyclic adenosine monophosphate-responsive element (CRE). E5-mediated EGFR activation was followed by the phosphorylation of EFGR's downstream signaling molecules PI3K/Akt and ERK/MAPK, inducing COX-2 expression. (Kim et al., 2009, Branca et al., 2004) So for E5 the COX-2 expression is induced through the EFGR-signaling pathway, increasing vascular endothelial growth factor (VEGF). This plays a central role in switching on the angiogenic phenotype during progression of cervical cancer. E5 upregulates the expression of COX-2 by increasing the COX-2 promoter activity. E5 enhances the transcription of COX-2 by AP-1 and much more important, by the activation of NF-kB, making CRE and NF-kB binding sites respectively the critical regulatory sites for the E5-induced COX-2 expression. The stimulation of the promoter activity was completely dependent on NF-kB-binding site and partially on the CRE-binding site. (Kim et al., 2009, Kim et al., 2006) E6 and E7 stimulate COX-2 transcription by enhancing the binding of activator protein-1 (AP-1) to cyclic adenosine monophosphate-responsive element (CRE), but not on a NF-kB dependent manner. (Subbaramaiah and Dannenberg, 2007) Targeting both EFGR and COX-2 may be an effective approach for the treatment of cervical cancer

The role of NF-kB in chemo- and radiotherapy resistance

NF-kB plays an important role in the resistance to chemo and radiotherapy. It is demonstrated that NF-κB is activated by ionizing radiation (IR), and NF-κB activity has been increased in different cell lines after exposure to cytotoxic agents (Kraus and Lis, 2003). Several molecular components and signalling events, including PTKs, PKC, ROS, RAS, ATM or IKK, were identified in the pathways where NF-kB activation by IR had been observed. Both high and low IR doses seem to invoke signalling events causing activation of NF-κB. It has been demonstrated that induction of inflammatory cytokines and molecules following IR exposure is coordinated by the activation of NF-Kb, both in vitro

and in vivo models (Criswell et al., 2003). This activation has been linked with resistance to chemotherapy and radiotherapy in several cell lines.

Ahmed et al (Ahmed and Li, 2008) described different mechanisms by which tumor cells develop adaptative response to therapeutic irradiation. As a part of a prosurvival signalling network overexpression of oncoprotein HER-2 in correlation with PI3K/Akt pathway activation is trigered, increasing cell growth and survival, which leads to NF-Kb activation. In addition, it has been described that this overexpression can increase the risk of local tumor relapse after radiation therapy in a group of patients. Wu et al (Wu et al., 2006) demonstrated that ATM (ataxia telangiectasia mutated), a DNA damage sensor, is involved in a signaling complex that promotes ubiquitination of NEMO, leading to NF-kB activation. Loss of ATM function appears to promote radiosensitivity in some cell lines. The mitochondrial antioxidant MnSOD (mitochondrial antioxidant manganese-containing superoxide dismutase) also plays a key role in this pathway. In fact, MnSOD overexpression allows tumor cells to avoid apoptosis by modulating ROS levels in the mitochondria, which promotes NF-kB activation. Suppression of this pathway induces adaptative radioresistance in preclinical models. Other important mediators are cell cyclin proteins, especially cyclin B1, which is related with radioresistant phenotype in several cell lines. In this way, radiosensization can be achieved at clinically relevant doses of irradiation by inhibiting NF-κB in many cancer cell types.

NF-kB is also involved in resistance to endocrine and chemotherapy. In vitro its activity is inversely correlated with cellular sensitivity to cytotoxic agents. Particularly platinum-based agents, anthracyclines and taxanes can promote activation of NF-kB pathway. It has been hypothesized that the induction of NF-kB by chemotherapy leads to a dysregulated apoptotic response, involving loss of mitochondrial function and death receptor signaling through activation of caspase cascade, resulting in a transcriptional regulation of apoptotic gene targets. Several factors, such as cell type, nature of stimulus and chromatin modifications, will define the pro or anti-apoptotic activities of NF-kB (Godwin et al., 2013). A dysregulated NF-kB/SNAIL/YY1/RKIP loop has recently been reported in the regulation of resistance to immunotherapy (Bonavida, 2014). The precise interaction between ER and NFκB and how this contributes to the attenuated responsiveness of ER positive tumors to hormonal treatment remains unclear (Sas et al., 2012). The inverse correlation between NF-kappaB activation and ER activation is due to EGFR and/or ErbB2 overexpression, resulting in NF-kappaB activation and ER downregulation (Van Laere et al., 2007).

Several studies have demonstrated that NF-κB inhibition results in the reversal of resistance to endocrine-, chemo and radiotherapy. One example is bortezomid, a proteasome inhibitor with NF-κB inhibition capacity in different cell tumors. This compound has demonstrated to increase apoptosis and reduce cell growth in combination with chemotherapy or IR compared with chemo or radiotherapy alone (Russo et al., 2001). Addition of a proteasome inhibitor to anti-hormonal therapy resulted in a clinical benefit rate of 22% in a limited number of patients with endocrine resistant and progressive metastatic breast cancer (Trinh et al., 2012). These data suggest that bortezomid could play an important role as chemo, endocrine or radiosensitizing agent. Sulfasalazine has also shown to inhibit NF-κB pathway, increasing sensitivity to cytotoxic agents and IR. IKK inhibitors, glucocorticoids, antisense RNA and inhibitory peptides have also been identified as NF-κB inhibitors.

NF-kB as a target for treatment

Taking into account the important role of NF-kB signalling in carcinogenesis and tumor progession, targeting NF-kB as systemic cancer therapy has been explored extensively (Table 3). Hundreds of compounds have been reported to inhibit NF-kB but their clinical efficacy has been disappointing up to now, except for certain types of lymphoma and leukemia (Xia et al., 2014). Most current NF-kB targeting strategies lack cancer cell specificity. A novel 4,6-substituted 1,2,-triazolo-1,3,4-thiadiazole was shown to inhibit invasion of cervical cancer SiHa cells and potentiates the apoptotic effect of TNFalpha by abrogating NF-kB activation cascade (Ningegowda et al., 2017). A recent study from Ethiraj et al showed synergistic inhibitory effects of interferon beta and low dose cisplatin on human cervical cancer cells. As interferon beta represses NF-kB/p-Akt signalling and increased PARP expression this suggests that the inhibition of the NF-kB/p-Akt signalling pathway may play a critical role in the anticancer effects of combination treatment along with the induction of PARP (Ethiraj et al., 2016). Deshpande et al demonstrated that alpha-linolenic acid could be explored for its therapeutic

potential in cervical cancer as is decreased the expression of NF-kB, COX2, c-JUN, pERK1/2 proteins, and reduced the expression of the HPV oncoproteins E6 and E7 resulting into restoration of the expression of the tumor suppressor proteins p53 and Rb (Deshpande et al., 2016).

Conclusion

The NF-kB pathway seems to be an important player in the development of cervical cancer. In the early stages of oncogenesis NF-kB is high-jacked by HPV to allow it to create a chronic inflammatory status. Due to a persistent HPV infection and mutational changes a tumor may emerge from a premalignant lesion and this seems to be accompanied by a progressive loss of responsiveness to the NFkB mediated growth inhibitory signal. As cervical cancer progresses the anti-proliferative functions of the NF-kB network are downregulated and it shows pro-tumorigenic effects. A significant linear relationship was seen between the increasing grade of CIN and the intensity of cytoplasmic NF-kB expression. This suggests a tumor-promoting role for NF-kB in cervical cancer. Further studies are needed to clarify if members of these pathways are of clinical interest as biomarkers or therapeutic targets.

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Table 1. Antiapoptotic pathways targeted by NF-Kb

Mechanism	Characteristics	Reference

Activation of STAT3	Positive feedback with NF-Kb activation Upregulation cell cycle and avoid cell death Regulation of c-myc, survivin, Bcl-xl, Cyclin 1	(Aggarwal et al., 2009)
Upregulation of survivin	Inhibition of caspases 3 and 7 Downregulation Fas-mediated apoptosis	(Jaiswal et al., 2015)
Induction of AID/APOBEC family	Induct mutations in p53 and c-myc Control DNA damage Upregulation to Mdm2	(Rebhandl et al., 2015)
Supression of death cell receptors	Downregulation of TRAIL receptors DR4 and DR5 Upregulation of FLIP, an inhibitor of protease-dependent activation of apoptosis Upregulation of DcR1 (competitor of death receptors)	(Bernard et al., 2001)
Activation of antioxidant enzymes	Upregulation on MnSOD and FHC Protection of ROS mediated apoptosis	(Ahmed and Li, 2008)
Inhibition of caspases	Supression of caspases 3,7 and 9 by the IAPs pathways Downregulation of caspase 8	(Wright et al., 2005)
Activation of Bcl-2 antiapoptotic members	Competitive inhibition of proapoptotic Bcl family members Development of Bcl-xL by PI3K/Akt pathway	(Chen et al., 2000)

Table 2. HPV Early Proteins and their cellular targets

HPV protein	Function	Targets

E1	Viral genome replication Induced NF-Kb activation	RPA Topoisomerase
E2	Viral DNA replication Viral DNA transcription Repression of E6 and E7 genes	Brd4, ChiR1
E4	Facilitate virion release and transmission Disregulation of cytokeratin network	Cytokeratin 8/18
E5	Mediates mitogenic signals of growth factors Activate EGFR and promote COX-2 expression Inhibition of inmune response	EGFR, MHC 1, TRAIL receptor, FAS receptor
E6	Regulation NF-kB expression Maintenance of viral genome together with E7 Deregulation cell cycle control Promote cell proliferation Block apoptosis	p53, p73, p300, IRF3, BAK, BAX, ADA3, CPB, TERT, MAGI-1, Caspase 8, c-Myc, PDZ domain proteins, Fibulin-1
E7	Regulation NF-kB expression Proliferation, inhibition of apoptosis Induction malignant transformation Reactivation of cellular replication mechanisms	pRb, HDAC, p21, p27, p107, p130, IRF-1, ATM, CDK/cyclin A and E, ATR, gamma-tubulin, TBP

Table 3. NF-Kb inhibitors which could develop chemosensibility or radiosensibility

Family	Members
Antioxidants	Disulfiram Curcumin Melatonin L-cystein Flavonoids
Proteasome inhibitors	Bortezomid Polyphenols Hydroxiureas Allosteric inhibitors
Non-steroidal antiinflamatories	Aspirine Sulindac Salicilates
Antiinflamatory drugs	Sulfasalazine
Glucocorticoids	Triamcinolone Clobetasol Dexametasone
IKKb Inhibitors	BA-Y11 PS-1145 Arsenic trioxide
Statins	Cerivastatin Lovastatin Simvastatin
Other compounds	Curcumin Capsaicin Melatonin Resverastrol