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Role of nuclear factor- κ B in melanoma

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Summary

Nuclear Factor-kappa B (NF- κ B) is an inducible transcription factor that regulates the expression of many genes involved in the immune response. Recently, NF- κ B activity has been shown to be upregulated in many cancers, including melanoma. Data indicate that the enhanced activation of NF- κ B may be due to deregulations in upstream signaling pathways such as Ras/Raf, PI3K/Akt, and NIK. Multiple studies have shown that NF- κ B is involved in the regulation of apoptosis, angiogenesis, and tumor cell invasion, all of which indicate the important role of NF- κ B in tumorigenesis. Thus, understanding the molecular mechanism of melanoma progression will aid in designing new therapeutic approaches for melanoma. In this review, the association between NF- κ B and melanoma tumorigenesis are discussed. Additionally, the potential of emerging selective NF- κ B inhibitors for the treatment of melanoma is reviewed.

Keywords

nuclear factor- κ B; melanoma; apoptosis; tumorigenesis; NF- κ B inhibitors

1. Introduction

Melanoma is the most aggressive form of skin cancer and its incidence has increased over 6-fold over the past 50 years. Metastatic melanoma is estimated to have caused 7600 deaths in 2003 and this disease is the second greatest cause of lost productive years among cancers [1, 2]. Although causality has been difficult to link in melanoma, sun exposure and genetic susceptibility are considered important predisposing factors. Traditionally, melanoma development and progression is viewed in five distinct stages. The common acquired nevus is the first step followed by dysplastic nevus showing an increased level of structural and architectural atypia. The third step is the first recognizable malignant stage, defined as the radial growth phase (RGP) primary melanoma. The cells in this phase are locally invasive but they lack metastatic capacity. Radial growth phase cells can progress to vertical growth phase (VGP) primary melanoma lesions, the fourth step in progression. In this step, melanoma cells infiltrate and invade the dermis as large clusters of cells and exhibit metastatic potential. Metastasis to distant organs followed by overgrowth of tumor cells at these sites is the final step in the progression process [3].

Although this model depicts melanoma progression as result of a series of genetic defects intrinsic to melanocytes, our current knowledge about the importance of the tumor microenvironment in control of growth, differentiation, invasion and metastasis adds to the complexity of the disease, hence the challenges faced both in clinics and research laboratories

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in the fight against melanoma. Due to the complex nature of the disease, melanoma has proven to be highly resistant to conventional chemotherapy with dacarbazine (DTIC) or its derivative Temozolomide (TMZ) having the best single agent activity with a response rate of only 15–20% and a short 4 month median response duration [4,5]. Patients at high risk for recurrence (stage III) are frequently given IFN- α as adjuvant treatment. Its effectiveness is widely debated, but even supporters acknowledge its benefit as small, accompanied by a large cost in toxicity [6]. Patients with metastatic disease (stage IV) have a median survival of 6–10 months with a 5-year survival of <5% [5]. Thus far, effective treatment options have been limited at best and it is imperative to investigate new therapeutic targets for the treatment of melanoma in order to improve the dismal prognosis for this disease. Recent advances in the understanding of the underlying biology in progression of melanoma have identified key signaling pathways that are important in promoting melanoma tumorigenesis, thus providing dynamic targets for therapy. One such important target identified in melanoma tumor progression is the nuclear factor-kappa B (NF- κ B) pathway [7,8]. In this review, the role of NF- κ B in melanoma tumorigenesis will be discussed and potential anti-tumor mechanisms of NF- κ B inhibitors will be evaluated.

2. Constitutive activation of NF- κ B in melanoma

Constitutive activation of NF- κ B is an emerging hallmark of various types of tumors including breast, colon, pancreatic, ovarian, and melanoma [9–14]. In the healthy human, NF- κ B regulates the expression of genes involved in normal immunologic reactions (e.g. generation of immunoregulatory molecules such as antibody light chains) in response to proinflammatory cytokines and by-products of microbial and viral infections [15–17]. NF- κ B also modulates the expression of factors responsible for growth as well as apoptosis. However, increased activation of NF- κ B results in enhanced expression of proinflammatory mediators, leading to acute inflammatory injury to lungs and other organs, and development of multiple organ dysfunctions as well as cancer.

The NF- κ B proteins constitute a family of proteins with homology to the chicken oncogene, *rel*. There are five known mammalian NF- κ B subunits, each characterized by ankyrin repeat elements: p65 (RelA), RelB, Rel (c-Rel), p50/p105, and p52/p100. The NF- κ B proteins share an approximately 300 amino acid N-terminal domain called the Rel homology (RH) domain containing sequences important for DNA binding, inhibitor of NF- κ B (I κ B) binding and dimerization. However, they differ in their C-terminal domain in that RelA, RelB, and cRel exhibit transactivating functions, while p100 and p105 contain inhibitory domains. Upon various stimulations, the latter are processed to shortened, active forms called p52 and p50, respectively [18]. The NF- κ B protein is composed of two subunits, which may vary, affecting the transcriptional activity of the protein. For example, p50 homodimers lack strong transactivation domains and can actually inhibit gene expression by competing with p65/p50 or other transactivating complexes for the κ B-sites. The most common Rel/NF- κ B dimer in mammals contains p50-RelA and is specifically called NF- κ B. For the purposes of this review, NF- κ B will be used to refer any induced complex that can be translocated from the cytoplasm to the nucleus and can bind to κ B-sites.

NF- κ B proteins are normally sequestered in the cytoplasm, due to the binding of I κ B to the nuclear localization sequence of NF- κ B complex. With activating signals there is phosphorylation, ubiquitination, and degradation of I κ B by the 26S proteasome, thus allowing the NF- κ B complex to translocate into the nucleus and bind specific DNA promoter sequences [19]. *In vitro* studies have shown that NF- κ B binding is constitutively elevated in human melanoma cultures compared to normal melanocytes [20,21]. This observation correlates with data from clinical specimens as well, where RelA expression is significantly elevated in human nevi and melanomas relative to normal skin. This elevation in expression also correlated with

increased phosphorylation and nuclear translocation of RelA [21,22]. Given that these NF- κ B aberrations are detected at early stage of melanoma tumorigenesis and are sustained throughout tumor progression, one could conclude that NF- κ B may play a pivotal role in many aspects of melanoma tumorigenesis.

3. Role of NF- κ B in melanoma tumor progression

Given that melanoma is a disorder that manifests diverse sets of clinical presentation and aggressiveness, NF- κ B is an ideal target since its constitutive activation may contribute to multiple steps in processes such as transformation, initiation, promotion, angiogenesis, invasion, and metastasis. The role of NF- κ B in these processes is discussed below.

3.1. Apoptosis resistance and cell proliferation

In processes such as tumor initiation and promotion where prolonged survival of cells is a crucial event, NF- κ B plays an important role as a mediator of inhibition of apoptosis. In melanoma, NF- κ B has been shown to activate expression of anti-apoptotic proteins such as tumor necrosis factor receptor-associated factor 1 (TRAF1), TRAF2, and the inhibitor-of-apoptosis (IAP) proteins c-IAP1, c-IAP2, and melanoma inhibitor of apoptosis (ML-IAP), survivin as well as Bcl-2 like proteins [23–28]. Indeed, expression of the IAP family members survivin and ML-IAP becomes detectable in melanocytic nevi and is further increased as melanoma progresses to invasive and metastatic phase [29–31]. Furthermore, tumour necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis is suppressed by NF- κ B-dependent transcription in tumor cell lines [27], including melanoma [32]. Alternatively, suppression of NF- κ B activity switches the prevailing death pathway in melanoma from Fas ligand (FasL) to TNF-mediated apoptosis [33]. In support of this concept, adenoviral transfer of I κ B to melanoma cells renders them sensitive to TNF induced cytotoxicity [34]. Using a cell culture system we have shown that delivery of the super-repressor form of I κ B to melanoma cells blocks focus formation and targets tumor cells to undergo apoptosis [35]. In addition to inhibition of apoptosis, NF- κ B activation may regulate cell cycle progression by controlling expression of important cell cycle regulatory proteins such as cyclin D1 and cyclin dependent kinase 2 (CDK2), further contributing to tumor growth [36–38]. These cell cycle regulatory proteins have been shown to be critical in the control of the G1/S transition and by overexpressing cyclin D1 and CDK2 among others, melanoma cells escape the cell-cycle control mechanisms and are able to initiate cell proliferation.

The constitutive nuclear activation of NF- κ B could play a major role in the endogenous expression of chemokines, interleukin (IL)-1 and IL-6, vascular endothelial growth factor (VEGF), as well as other factors reported to impact melanoma growth. We have observed that once CXCL1 and CXCL8 are induced, these secreted chemokines feed back on the activation of NF- κ B and further perpetuate the cycle. Endogenous activation of NF- κ B in melanoma tumor cells can be inhibited ~50% with antibody to CXCL1, suggesting that additional cytokines or alterations in the signal transduction pathway contribute to the dysregulation of NF- κ B in melanoma [39]. Furthermore, CXCL8 expression in human melanoma cells correlates with the level of anoxia and the aggressiveness of the melanoma. The induction of transcription of CXCL8 in these necrotic/anoxic areas of the tumor is dependent upon NF- κ B and AP1 activity [40]. Transfection of nonmetastatic and CXCL8-negative melanoma cells with the CXCL8 gene render them highly tumorigenic and increases their metastatic potential through upregulation of MMP-2 expression and activity, as demonstrated by increased invasiveness through Matrigel-coated filters [41]. In addition to tumor growth and invasion, these chemokines along with VEGF are involved in promotion of angiogenesis in melanoma as well and may actually work in concert with each other to promote neovascularization in tumors [42]. It was recently reported that tumor vascularity represents an early requirement for melanoma progression and that NF- κ B acts as a mediator between melanoma cells and

tumor vasculature [43]. Taken together, these data support a role for endogenous activation of NF- κ B in association with progression and enhanced metastatic potential of malignant melanoma cells and suggest that targeting NF- κ B may have significant therapeutic effect in clinical trials.

3.2. Invasion and metastasis

In invasion and metastasis of melanoma, NF- κ B may regulate the production of prostaglandins via cyclooxygenase-2 (COX-2), which has been shown to be overexpressed in melanoma [44,45]. It was shown that COX-2 is expressed in the majority of primary malignant melanoma, as well as in five human malignant melanoma cell lines. These cell lines produced PGE₂, which could be inhibited by the specific COX-2 inhibitor, NS-398. Although, this inhibition of COX-2 did not change proliferation of malignant melanoma, it reduced matrigel invasion in all cell lines. These data prompt for further investigation in the function of COX-2 in malignant melanoma tumors in determining the indications for chemoprevention.

NF- κ B may also facilitate invasion and metastasis by inducing expression of molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), endothelial-leukocyte adhesion molecule-1 (ELAM-1), and matrix metalloproteinases (MMPs) [46–50]. These molecules are mediators of migration of cancerous cells and crossing of vessel walls and invasion at sites of metastasis. Screening of progressive melanoma lesions has demonstrated that the percentage of melanoma cells expressing ICAM-1 increases directly with tumor thickness and is higher in metastasis than in primary lesions. It has been postulated that ICAM-1 may facilitate the interaction between melanoma cells and tumor infiltrating leukocytes, thereby enhancing tumor cell adhesion to migratory and invasive leukocytes, enabling individual cells to dissociate from the primary tumor [51–53].

VCAM-1 appears to contribute to melanoma metastasis by aiding in cell adhesion at sites of metastasis. Langley et al. show that upregulation of VCAM-1 is not a prerequisite for the formation of pulmonary metastasis during spontaneous melanoma metastasis, but exhibits organ-specific enhancement once lung metastasis become well established. Furthermore, these organ-specific increases in VCAM-1 expression correspond with documented clinical patterns of melanoma metastasis and are independent of systemic levels of TNF- α and IL-1 α . However, increased VCAM-1 may be the result of melanoma-induced alterations at the local level, based upon evidence of melanoma cell occupation in heart, brain, and liver in mice with pulmonary metastasis [54].

The expression of ELAM-1 (E-selectin) plays a critical role in facilitating leukocyte adhesion to and subsequent transmigration of endothelium. On this basis, E-selectin has been suggested to promote tumor cell attachment to endothelium, thereby facilitating metastasis of certain types of tumors. Expression of E-selectin was shown to redirect metastasis of tumor cells expressing appropriate ligands *in vivo* [55]. The role of MMPs in melanoma progression, however, appears to be at an early stage, evidenced in a study by van den Oord et al. malignant melanoma lesions with thickness <1.6 mm, 63% expressed gelatinase B (MMP-9), whereas in melanoma lesions with >1.6 mm thickness, only 10% expressed MMP-9, indicating that early invasion of malignant melanoma is associated with de novo expression of MMP-9 by neoplastic melanocytes and this expression of MMP-9 may be partly responsible for the stromal changes observed in thin malignant melanoma. The absence of MMP-9 in the vertical growth phase and in metastatic lesions suggests that other factors are involved in tissue degradation and remodeling during later stages of tumor progression in malignant melanoma [56]. Thus, NF- κ B may be a central regulator at multiple stages in the progression of malignant melanoma.

In addition to its role in protection against apoptosis, NF- κ B may also play an critical role in resistance to conventional chemotherapy [57] by inducing expression of ATP-binding cassette

(ABC) transporters [58,59]. Drug resistance is the major reason for melanoma therapy failure and melanoma cells often develop multiple mechanisms of drug resistance during tumor progression. Overexpression of multidrug resistance genes and their encoded P-glycoproteins is a major mechanism for the development of multidrug resistance in cancer cells. NF- κ B has been shown to be a transcriptional regulator of the expression of these genes [58–60]. Recent data indicate that NF- κ B activity and NF- κ B p65 subunit level were constitutively higher in MDR human leukemia and mouse mammary carcinoma cells than in drug-sensitive parental cells. Inhibition of NF- κ B resulted in increased cytotoxicity of anticancer drugs, with consequent reversal of the drug-resistance of MDR cells [61]. These findings suggest yet another means by which NF- κ B may facilitate melanoma tumor protection and as such further establish the importance of NF- κ B as a target for the treatment of the disease.

4. Mechanism for deregulation of NF- κ B

The deregulation of NF- κ B in hematopoietic tumors as well as solid tumors has gradually become established as a paradigm [19]. Increase in p50 or p100 (the precursor of p52) was reported in non-small cell lung carcinoma and human breast carcinoma biopsies as well as head and neck cancer cells [10,12,62,63]. Nuclear localization of NF- κ B associated with breast cancer may be related to the escape of these tumor cells from apoptosis [11]. This alteration in NF- κ B is often accompanied by increased expression of anti-apoptotic cytokines and chemokines, growth factors, and oncogenes [35,64–70], adhesion proteins and proteases [71], as well as inhibitors of apoptosis [72].

Inducible pathways for regulation of these events involve activation of the I κ B kinase complex (comprised of IKK α , IKK β and the adaptor molecule known as IKK γ or NEMO), leading to the phosphorylation of specific serine residues of I κ B (S32 and S36 of I κ B α , or S19 and S23 of I κ B β). In addition, there are non-inducible pathways for ubiquitination and degradation of I κ B, which result in the targeting of this protein for degradation in the proteasome [73].

Malignant melanoma cells exhibit a constitutive activation of IKK, resulting in more rapid degradation of I κ B, nuclear localization of NF- κ B, and enhanced transactivating capacity of the NF- κ B complex [21,39,74]. Embryonic fibroblasts from both IKK β and IKK α null mice are unable to induce NF- κ B transactivation. However, IKK β restoration in IKK $\alpha\beta$ null mice will not restore cytokine-stimulated activation of NF- κ B. Both IKK α and IKK β are required for induction of the transactivating activity of NF- κ B [75]. Since activation of NF- κ B induces the transcription of inhibitors of apoptosis as well as factors associated with tumor angiogenesis, metastasis, and growth, we have come to understand that NF- κ B could be an important potential therapeutic target in cancer, particularly if we could identify which upstream kinases affecting NF- κ B become deregulated during tumorigenesis (Figure 1).

4.1. NIK modulation of NF- κ B

Several recent studies have suggested that mitogen-activated kinases (MAPKs) such as NF- κ B-inducing kinase (NIK) and MAP-kinase-kinase 1 (MEKK1) can participate in the activation of NF- κ B in the cytoplasm as well as in the modulation of its transactivation potential in the nucleus. NIK was initially thought to preferentially phosphorylate and activate IKK α , while MEKK1 was proposed to preferentially phosphorylate and activate IKK β [76,77]. Interestingly, recent work with the NIK and MEKK1 $-/-$ mice suggest that NIK and MEKK1 are not essential for TNF α induction of NF- κ B, but are essential for activation of NF- κ B by other factors. For example, NIK is required for lymphotoxin- β activation of NF- κ B [78].

NIK was first identified on the basis of its association with the TRAF2 [79]. NIK associates with and is reported to activate the phosphorylation of IKK α and IKK β . When over-expressed, NIK enhances NF- κ B activity and expression of kinase-defective NIK blocks NF- κ B activation

in response to most inducers. NIK is involved in the processing of p100 to the NF- κ B/p52 subunit [80]. This p52 subunit will either homodimerize, interact with p65 to form the NF- κ B p65/p52 heterodimer or form a heterodimer with RelB. Thus, the enhanced NIK activity in tumors [21] could be associated with the enhanced level of nuclear p52, which was reported to be present in breast cancer [81].

Disregulation of NF- κ B inducing kinase (NIK) has been shown to play a role in the constitutive NF- κ B activity in melanoma. Data suggest that IKK-associated NIK activity is enhanced in melanoma cell lines compared with normal human epidermal melanocytes (NHEMs) [21]. Kinase dead NIK blocks constitutive NF- κ B or CXCL1 promoter activity in melanoma cells, but not in control NHEMs, while transient overexpression of wild type NIK results in increased phosphorylation of extracellular signal-regulated kinases 1 and 2 (ERK1/2). This phosphorylation is inhibited in cells expressing the kinase-dead NIK. Moreover, the NF- κ B promoter activity in melanoma cells decreased with over expression of dominant negative ERK expression constructs [21]. These data support the hypothesis that NIK mediated ERK activation contributes to the NF- κ B activity.

4.2. AKT modulates NF- κ B transactivation

Once I κ B is degraded by the proteasome, the nuclear localization sequence of the p50/p65 or p52/p65 NF- κ B complex is exposed and consequently this complex can move to the nucleus and bind to the NF- κ B element of the promoter of responsive genes. However, simply binding to the DNA is not sufficient. The p65 needs to be phosphorylated in the transactivation domain to be fully active. It has recently been suggested that a serine/threonine protein kinase isolated from the AKR mouse thymoma (AKT) may facilitate the phosphorylation of p65 on serines 529 and 536 [82–85]. In response to activation of PI3 kinase, AKT is recruited to the membrane by phosphatidyl inositol (3,4,5) trisphosphate or phosphatidyl inositol (3,4) bisphosphate, and becomes phosphorylated by 3'-phosphoinositide-dependent-kinase-1 (PDK-1) on Threonine-308 in the kinase domain and by phosphoinositide-dependent-kinase-2 (PDK-2) on Serine-473. AKT phosphorylates a number of substrates at a RXRXXS/T motif, some of which mediate escape from apoptosis and contribute to cell survival. One such substrate identified is IKK α , which has a perfect consensus sequence for AKT, and Li and Stark have suggested that AKT activation of IKK α could be upstream of the phosphorylation of p65 by IKK [82]. However, work from Delhase and Karin does not support this possibility [83]. The authors argue that since IKK- β can be fully activated by TNF- α or IL-1 in IKK- α deficient cells, IKK- α phosphorylation by Akt or any other kinase is not essential for IKK activation and subsequent NF- κ B induction. However, one should be careful interpreting data from knock-out systems, since compensation phenomenon in these cells can not be excluded. Other work in the field showed that the phosphorylation of the p50 NF- κ B subunit by the PI3K/Akt pathway increases the binding of the NF- κ B complex to DNA [84]. Moreover, inhibitors of PI3K blocked the endogenous NF- κ B luciferase activity of malignant melanoma cells, though these inhibitors did not block IKK phosphorylation of the I κ B α substrate [86], thus indicating Akt-mediated NF- κ B activation is downstream of IKK or separate from the IKK α mediated phosphorylation of I κ B α .

One critical regulatory molecule in the PI3K/Akt pathway is the lipid phosphatase known as phosphatase and tensin homolog (PTEN), which is known to shut off AKT activation by dephosphorylating phosphatidylinositol-3,4,5-trisphosphate (PIP3) and blocking AKT membrane localization. PTEN is also reported to block NF- κ B activation without altering the I κ B degradation pathway [84]. However, PTEN is often deleted or mutated in melanoma tumors [87,88]. The activation of AKT, which we observe in some human melanoma cells *in vitro* is accompanied by loss or reduction in PTEN [86]. Furthermore, absence of PTEN was observed in a significant proportion of primary cutaneous melanoma supporting a role for

PTEN loss in the pathogenesis of melanoma [89]. Loss of PTEN with consequent enhanced activation of AKT has also been reported for a number of other tumor types, suggesting this is a frequent step associated with transformation.

4.3. Ras/Raf activation of NF- κ B

The activation of NF- κ B can suppress apoptosis induced by oncogenic ras [90]. Paradoxically, when cells are transformed by Ha-Ras, NF- κ B is activated [91]. Ras has been shown to utilize Raf-dependent and independent pathways to activate NF- κ B transcriptional activity and this NF- κ B activity is required for oncogenic Ras to transform NIH 3T3 cells [91,92]. Cells expressing H-Ras has been shown to exhibit NF- κ B activity that correlates with sustained IKK activation and lower steady-state levels of I κ B in the cytosol. The activation of NF- κ B in these cells was able to impair staurosporine-induced apoptosis, thus indicating that Ras activation of NF- κ B contributes to protection of cells against apoptosis [93].

Whereas about 20% of melanoma appear to carry mutation in the N-Ras oncogene [94,95], over 60% were recently reported to contain mutation within the B-Raf gene [96,97]. Mutation in B-Raf resulting in the V600E amino-acid substitution was found in 41 of 60 (68%) melanoma metastases, 4 of 5 (80%) primary melanomas and, unexpectedly, in 63 of 77 (82%) nevi, suggesting that mutational activation of the RAS/RAF/MAPK pathway in nevi is a critical step in the initiation of melanocytic neoplasia [96]. The mutations in Ras and B-Raf render both kinases constitutively active, thereby eliciting constant activation of down-stream signaling components and the corresponding transcription factor substrates, including NF- κ B, c-Jun and activating transcription factor 2 (ATF2). Since pathways such as Ras/Raf may impinge on the NF- κ B pathway to further activate NF- κ B, targeting the Ras/Raf pathway may offer a potential mechanism for therapeutic targeting of NF- κ B in melanoma.

4.4. Other factors can also modulate NF- κ B

While many kinases have been demonstrated to contribute to the activation of NF- κ B, it has been difficult to clearly demonstrate the requirement for an activating kinase upstream of the IKK complex. NIK is a MAP3K, as are TAK1, MEKK1 and MEKK3 ([82], for review). Deletion of NIK and MEKK1 did not block TNF α or IL-1 mediated induction of IKK activity, leaving TAK1 and MEKK3 open as potential modulators of IL-1 and TNF α induction of IKK. In addition to the MAP3 kinases and AKT, a number of other indirect modulators of NF- κ B activation have been described [92,98–102]. Protein kinase C (PKC) has also been noted as a potential modulator of IKK activity and PKC activation of IKK is linked to tumorigenesis [103–107]. In addition to IKK phosphorylation and activation, the transactivation potential of NF- κ B can be induced by phosphorylation of p65. Inducible p65 phosphorylation can occur at serine 529 by casein kinase II (CKII) [108,109], serine 536 by the IKK complex [110] and at serine 276 by PKAc as well as MSK1 [111–113]. This p65 phosphorylation event may also be mediated by IKK β [110] or possibly by IKK α [75]. Thus, in trying to discern why there is dysregulation of NF- κ B in tumor cells, it is important to look at a number of upstream signal transduction pathways.

5. Inhibitors of NF- κ B as melanoma therapy

With the slow progress in finding effective treatment of melanoma in recent years, the expectation is that novel treatment agents that target signaling pathways essential for melanoma growth and invasion may offer hope for an otherwise dismal disease. It is now clear that melanoma exhibit constitutive activation of NF- κ B. This activation of NF- κ B leads to endogenous expression of a number of factors associated with escape from apoptosis, tumorigenesis, and metastasis. Since the expression of many chemokines is modulated by NF- κ B, inhibition of NF- κ B offers the risk of inhibiting the migration of natural killer (NK) cells,

tumor infiltrating lymphocytes (TILs) and dendritic cells into the developing tumor and may therefore abrogate the host immune response against the tumor. However, this negative factor is apparently offset by the enhanced apoptosis of the tumor cells due to the inhibition of NF- κ B. Indeed, treatment of melanoma in mice with non-steroidal anti-inflammatory drugs (NSAIDs) such as sulindac has been shown to both inhibit NF- κ B and tumor growth, without detrimental effects on the host [19]. Thus, reagents like sulindac or other NSAIDs offer potential therapeutic reagents for human tumors [113–116]. Another class of NF- κ B pathway inhibitors identified is the proteasome inhibitors. Initial preclinical and clinical data with the proteasome inhibitor VELCADE (formerly known as PS-341) indicate it will provide a powerful means of killing tumor cells by targeting them toward apoptosis [117–119]. Indeed, combination treatment with VELCADE and TMZ resulted in complete response and remission of human melanoma tumor xenografts in nude mice [120]. Among natural inhibitors of NF- κ B pathway, Curcumin, the active ingredient from the spice turmeric (*Curcuma longa* Linn), has been shown to possess anti-oxidant and anti-inflammatory qualities. It has recently been demonstrated to possess anti-angiogenic effect and anti-metastatic property in mouse melanoma tumor models. Treatment of B16F10 tumors with Curcumin significantly inhibited MMP-2 activity, resulting in reduced tumor growth in this model [121,122].

The anti-tumor activity of nitrosylcobalamin (NO-Cbl) was recently demonstrated in melanoma by Chawla-Sarkar et al. NO-Cbl is an analog of vitamin B12 that delivers nitric oxide (NO) and increases the expression of tumor necrosis factor-related apoptosis-inducing ligand (Apo2L/TRAIL) and its receptors in targeted cells. The authors showed that NO-Cbl suppressed Apo2L/TRAIL- and TNF- α -mediated activation of a transfected NF- κ B-driven luciferase reporter. In addition, XIAP, an inhibitor of apoptosis, was inactivated by NO-Cbl and NO-Cbl treatment, rendering Apo2L/TRAIL-resistant malignancies sensitive to the anti-tumor effects of Apo2L/TRAIL *in vitro* and *in vivo* [123]. Thus, the use of NO-Cbl and Apo2L/TRAIL exploits the tumor-specific properties of both agents and represents another promising anti-cancer combination in the treatment of melanoma.

A major concern about utilizing the above mentioned inhibitors of NF- κ B is the lack of selectivity. Most of these agents target other key regulatory molecules along with NF- κ B, rendering it difficult to discern the specific role of NF- κ B in the progression of the disease and its attribute as a therapeutic target. Recent progress in developing specific inhibitors of NF- κ B, however, has resulted in designing inhibitors of IKKs. We demonstrated that treatment of melanoma cells with the specific IKK inhibitor, BMS-345541, reduced constitutive IKK activity in a reversible manner and blocked nuclear translocation of NF- κ B/p65. BMS-345541 treatment also resulted in effective blockage of hyper-proliferation of melanoma cells *in vitro* and melanoma tumorigenesis *in vivo*. BMS-345541 was capable of inducing melanoma apoptosis through down-regulation of IKK activity and suppression of NF- κ B-dependent transcription in melanoma. BMS-345541 treatment also contributed to the apoptotic process. However, the apoptotic effect of BMS-345541 was not mediated through the ERK1/2 signal transduction pathway. Alternate methods of inhibiting NF- κ B activity by over-expressing a dominant negative IKK β or a super-repressor form of I κ B α also induced melanoma cell apoptosis (Yang et al.- submitted manuscript). These data argue for the powerful role of NF- κ B in the future of melanoma therapy targets and support the notion of the recent trend observed in the therapeutics development field to use biologically based target.

6. Conclusions

Constitutive activation of NF- κ B is an emerging hallmark of various types of tumors and many experimental models *in vitro* and in animals indicate the role of this transcription factor in the regulation of apoptosis, tumor angiogenesis and proliferation, as well as tumor cell invasion and metastasis. The enhanced activation of NF- κ B in tumors appears to be partially due to

deregulation of upstream kinases such as Ras, Raf, NIK, and AKT that impinge on the NF- κ B pathway. Thus, NF- κ B may prove to be a key effector molecule executing the commands of top officials in above-mentioned signaling pathways. The importance of NF- κ B in melanoma tumor progression is evident in many recent studies utilizing various inhibitors of NF- κ B for the treatment of melanoma. The use of NF- κ B inhibitors has resulted in significant anti-tumor effects in melanoma tumor xenograft models and some have led to ongoing clinical trials. As we are eagerly awaiting the results of these studies, it's important to note that NF- κ B targeting for treatment of cancers such as melanoma is a rather new field and in need of optimization in both drug design as well as drug delivery in patients.

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Figure 1.
Signaling Pathways activating NF-κB and potential mechanisms by which NF-κB activation promotes melanoma development and progression.