17 NF-κB as a Molecular Target in the Therapy of Pancreatic Carcinoma

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Recent Results in Cancer Research, Vol. 177

Abstract

The constitutive activation of the transcription factor nuclear-factor kappa B (NF-κB) is a hallmark of many highly malignant tumours such as the pancreatic ductal adenocarcinoma and accounts for profound chemoresistance. Inhibition of NF-κB activation has been shown to be a useful strategy for increasing the sensitivity towards cytostatic drug treatment in vitro and in vivo. Moreover, various pharmacological substances (e.g. thalidomide, bortezomib, sulphasalazine) have already entered clinical studies partially showing promising results for certain types of cancer. Further studies will be needed, in particular for pancreatic ductal adenocarcinoma, to evaluate the therapeutic efficacy of appropriate combinations of a NF-κB inhibitor and cytostatic drugs.

17.1 Introduction

The conservative treatment of pancreatic cancer still proves to be quite difficult and poorly effective due to the broad resistance of this carcinoma to any kind of cytostatic drug therapy. One factor which has gained much attention during the last couple of years is the transcription factor nuclear factor kappa B (NF-κB) which has been recognized as a central determinant in the induction and manifestation of chemoresistance in pancreatic carcinoma cells. Accordingly, the pharmacological inhibition of NF-κB represents a plausible strategy to efficiently sensitize chemoresistant tumour cells towards cytostatic drug treatment.

17.2 Regulation and Function of NF-κB

The ubiquitous transcription factor NF-κB comprises hetero- and homodimeric protein complexes composed of members of the Rel/NF-κB protein family: RelA/p65, c-Rel, RelB, p50/NF-κB1 and p52/NF-κB2. Most abundant are the heterodimer RelA/NF-κB1 (p65/p50) and the homodimer NF-κB1/NF-κB1 (p50/p50), the former being transcriptionally active in many cell types. In non-stimulated cells, NF-κB is retained in the cytoplasm by inhibitory proteins of the inhibitor of NF-κB (IκB) family thereby masking the NF-κB nuclear localization domain and inhibiting its DNA binding activity (Fig. 17.1). Upon the canonical ("classical") activation by a great variety of cellular stimuli, i.e. cytokines, growth factors or viral proteins, the IκB kinase (IKK) complex– composed of the catalytic subunits IKK-α and IKK-β and the regulatory subunit IKK-γ/NF-κB essential modulator (NEMO)—becomes activated and subsequently phosphorylates IκB proteins. Then IκB proteins are subject to rapid polyubiquitination, followed by degradation by the 26S-proteasome. Thereby, NF-κB becomes released from IκB and translocates into the nucleus where it exerts its action as a transcription factor and, possibly, other yet-to-be-defined functions [1]. One the one hand, activation of NF-κB can be induced by different stimuli, and on the other hand, it is involved in the regulation of a multitude of genes amongst them those encoding for cytokines, growth factors, and anti- and proapoptotic proteins [1, 2]. Thus, NF-κB is an important regulator of cellular processes such as proliferation, apoptosis, cell growth and differentiation. Therefore, under

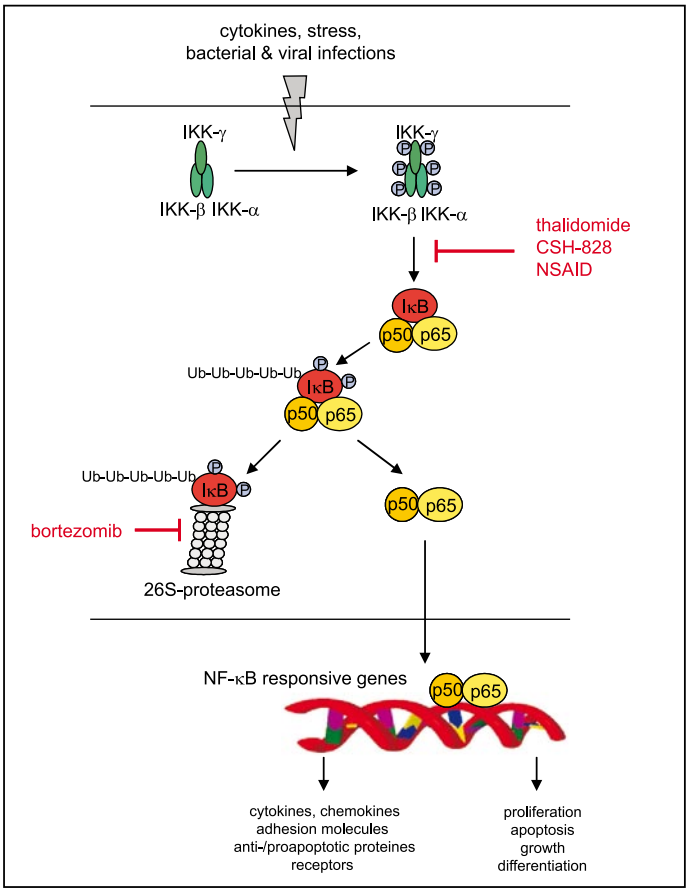

Fig. 17.1 Activation and inhibition of NF-κB. In non-stimulated cells, NF-κB (here represented as the heterodimer p50/p65) is inactive and sequestered in the cytoplasm by its inhibitor IκB. Cytokines, cellular stress, and bacterial and viral infections lead to the phosphorylation and, thereby, to the activation of the IKK complex (IKK-α, IKK-β, IKK-γ), which in turn phosphorylates IκB proteins. After additional polyubiquitination, IκB becomes degraded by the 26S-proteasome. Thus, NF-κB, released from IκB, is able to translocate into the nucleus leading to the expression of NF-κB responsive genes. Since many different genes are regulated by NF-κB, this transcription factor is essential for the control of cellular processes such as proliferation, apoptosis, cell growth and differentiation under physiological as well as under pathological conditions. NF-κB inhibitors being under clinical investigation, they either block IKK activity (thalidomide, CSH-828, non-steroidal anti-inflammatory drugs, NSAIDs) or the 26S-proteasome (bortezomib)

physiological conditions induction and activation of NF-κB is of vital importance in immune and inflammatory responses as well as in cellular homeostasis and organogenesis [3].

17.3 Constitutive Activation of NF-κB and Its Role in Carcinogenesis

Besides its fundamental role in many physiological conditions, NF-κB is also involved in pathological conditions such as chronic inflammation and carcinogenesis [4, 5]. Constitutive activation of NF-κB has been observed in haematological tumour diseases [6] as well as in various solid tumours, e.g. in melanoma [7] and carcinoma of the mamma [8], the colon [9], the prostate [10] and the pancreas [11, 12]. Aetiologically, constitutive activation of NF-κB in tumours can occur due to various conditions and factors. First of all, chronic bacterial or viral infections being major risk factors for various types of cancer, they can induce permanent NF-κB activation [1]. Furthermore, pro-inflammatory cytokines such as interleukin (IL)-1β [13], IL-1α [14] and tumour necrosis factor (TNF)-α [15] either released by immune cells or by other adjacent stromal cells might lead to constitutive nuclear translocation and DNA binding activity of NF-κB. Since some of these "inducer" cytokines are NF-κB target genes at the same time, an autocrine or paracrine amplification loop emerges leading to the constitutive cytokine-driven NF-κB activation, e.g. in pancreatic carcinoma cells [13]. Point mutation of the k-ras oncogene, which is a common and early event in the carcinogenesis of pancreatic

cancer, might also result in an enduring activation of NF-κB [11, 16]. In addition, overexpression and activation of the epidermal growth factor (EGF) receptor might contribute to tumour progression and an invasive phenotype of pancreatic cancer by permanent activation of NF-κB [17, 18]. Finally, chromosomal aberrations, e.g. in the genes *c-rel*, *rela*, *nfκb1*, *nfκb2* or *iκbα* have been found in haematological as well as in solid tumours, affecting expression or function of NF-κB directly or indirectly via alterations of IκB [19].

This constitutive NF-κB nuclear translocation results in the activation of a number of different genes leading to a permanently increased expression of pro-inflammatory and pro-oncogenic proteins such as inducible nitric oxide synthetase (iNOS), IL-1β, IL-8 or cyclin D1 [1, 4, 20]. Overexpression of the latter protein has been shown to promote cell survival and cell growth. Furthermore, constitutive NF-κB activation also contributes to tumour growth and tumour aggressiveness by increasing the angiogenic and invasive potential of tumour cells via increased expression of proangiogenic factors such as vascular endothelial growth factor (VEGF) and IL-8 [2, 10]. However, the most important tumourigenic mechanism by which NF-κB promotes tumour cell growth and carcinogenesis is the inhibition of programmed cell death (apoptosis) thus enabling propagation of genetically altered cells. The efficient prevention of apoptosis provided by NF-κB activity also implicates the most effective mechanism of tumour cells to gain protection from cytostatic drug treatment.

17.4 NF-κB as Determinant of Chemoresistance

While there are some reports indicating a rather apoptosis-promoting role for NF-κB [21–23], the majority of studies demonstrate NF-κB as a potent apoptosis suppressor. Although the crucial role of NF-κB in the protection notably from TNF-α and chemotherapeutic drug-induced apoptosis is widely proved, the exact mechanisms by which apoptosis prevention occurs is only now beginning to emerge. Several genes that certainly play a role in apoptosis inhibition, and whose expression is regulated by NF-κB, have been already identified. Thus, activation of NF-κB leads either to the up-regulation of anti-apoptotic genes or to the down-regulation of apoptotic genes. A prevalent mechanism by which activated NF-κB induces chemoresistance is the increased expression of cellular inhibitors of apoptosis (cIAP1, cIAP2, TRAF1, TRAF2, survivin) or the increased expression of the pro-survival bcl-2 homologue Bfl-1/A1 or of bcl-x(L) [24–27]. Increased expression of either apoptosis inhibitor that has been found in several tumour entities, e.g. in pancreatic carcinoma, leads to the disruption of caspase activation and thereby to the failure of apoptosis execution. Another mechanism by which activated NF-κB mediates resistance to chemotherapeutic drugs in pancreatic carcinoma cells represents the direct inactivation of caspases [28]. As a result of an IL-1β-driven constitutive activation of NF-κB, expression of inducible NOS (iNOS) and subsequently the release of nitric oxide (NO) are enhanced, leading to the inactivation of a broad spectrum of caspases. This efficient NO-mediated caspase inhibition obviously occurs via nitrosylation of certain cystein residues in the active site of the caspases [28]. In conclusion, NF-κB-mediated chemoresistance can be induced either by intrinsic mechanisms—e.g. by chromosomal aberrations or by interactions of tumour cells with adjacent stromal cells (fibroblasts, endothelial or immune cells)—or by extrinsic mechanisms, e.g. during a course of chemotherapy. Thus, constitutive activation of NF-κB significantly accounts for the pre-existing as well as for the acquired chemoresistance of pancreatic carcinoma cells [28, 29].

17.5 Inhibition of NF-κB as Strategy for Chemosensitization

In 1996 already, Wang et al. reported on the potential of NF-κB inhibition for improving the efficacy of cancer therapies [15]. Since chemoresistance of various tumours depends on the constitutive activation of NF-κB, a multitude of strategies has been developed and verified to prevent the activation or transcriptional activity of NF-κB, thereby enhancing chemosensitivity.

Three main strategies exist to inhibit NF-κB activation and function:

1. Inhibition of NF-κB protein expression
2. Interference with DNA binding of NF-κB
3. Inhibition of NF-κB activation

17.5.1 Inhibition of NF-κB Protein Expression

Guo et al. demonstrated that NF-κB protein expression blocking can be effectively achieved by delivery of p65-specific small interfering RNA (siRNA) to tumour cells in vivo indicating that inhibition of NF-κB activity by siRNA may have therapeutic potential [30]. Despite these promising findings, this technology requires significant improvement with respect to efficiency of delivery, duration of action and improved specificity and safety, before clinical application can be considered [31, 32].

17.5.2 Interference with DNA Binding of NF-κB

Some inhibitors such as Evans blue, Gallic acid and coumarin, and the novel quinone derivative E3330 have been shown to inhibit binding of the NF-κB subunit p50 to the DNA [33], but the exact mechanism of action remains to be elucidated. Further approaches may be the development and the design of ligands binding either to the κB site of the DNA or directly to the DNA binding sequence of the NF-κB protein. Although this can be accomplished by use of decoy κB sites or their analogues, such molecules might be quite large and polar, thus hampering their cellular uptake and bioavailability [34].

17.5.3 Inhibition of NF-κB Activation

The third and most advanced strategy of NF-κB inhibition—interference with its activation at different points of the activation signalling cascade—has already proved to be feasible and, most notably, to overcome chemoresistance in various tumour entities [34]. First of all, inhibitors of the 26S-proteasome (Fig. 17.1) have been shown to prevent NF-κB nuclear translocation and activity by inhibiting IκB degradation [35, 36]. To date, one proteasome inhibitor [PS-341, bortezomib, Velcade (Janssen-Cilag International, Beerse, Belgium)] has entered clinical application and will be discussed below in more detail. However, therapeutic effects seen after treatment with a proteasome inhibitor cannot only be attributed to inhibition of IκB degradation (and NF-κB activation) but also to the inhibited degradation of other proteins. Beside the low specificity of proteasome inhibitors, it has to take into consideration that abrogation of proteasomal degradation might also lead to the accumulation of proteins such as β-catenin, which can rather promote than suppress carcinogenesis [37].

Beside three recent publications describing IKK activity in a NF-κB-independent manner in Drosophila [38–40], there is little evidence that either IKKα or IKKβ phosphorylate proteins that are not involved in NF-κB signalling. Thus, the most effective and selective approach for blocking NF-κB activation might be given by inhibition of the IKKs (Fig. 17.1). So far, three main groups of agents exist that specifically inhibit IKK activity: immunomodulatory drugs (e.g. thalidomide and its derivates, flavonoids and cyclopentenone prostaglandins), the non-steroidal anti-inflammatory drugs (NSAID) including aspirin and salicylates, sulindac and its analogues, sulphasalazine and its metabolites and newly developed selective IKK inhibitors (e.g. CHS-828) [34]. Since thalidomide, sulphasalazine and CHS-828 have already been applied in preclinical as well as in clinical studies, the therapeutic potential of these substances in the treatment of chemoresistant tumour diseases can be evaluated soonest and is therefore described in more detail below.

17.5.3.1 Inhibition of NF-κB Activation by Blocking Proteasome Activity

Bortezomib

The therapeutic efficacy of the proteasome inhibitor bortezomib was broadly investigated in vitro and in vivo in different experimental settings using cells of prostate carcinoma [41], colorectal carcinoma [42], melanoma [43] or non-small cell lung carcinoma [44]. Bortezomib sensitizes tu-

mour cells to apoptosis induced by camptothecin CPT-11, gemcitabine or temozolomide [42–44]. Moreover, treatment with bortezomib alone already induces growth arrest and apoptosis which can be potentiated by co-treatment with chemotherapeutic drugs [41]. Fahy et al. demonstrated that bortezomib increases sensitivity to apoptosis-inducing agents by down-regulation of bcl-2 [45]. In androgen-dependent human prostate LNCaP cancer cells, bortezomib-induced growth arrest and apoptosis is accompanied by markedly elevated levels of p21(waf1) and p53 [41]. Most studies were performed in models of multiple myeloma showing that bortezomib decreases the apoptotic threshold to chemotherapeutic drugs such as doxorubicin and melphalan in multiple myeloma cell lines or even reverses chemoresistance in cells from multiple myeloma patients [46, 47]. As shown by gene expression profiling, bortezomib treatment results in the down-regulation of several effectors mediating a protective cellular response to genotoxic stress (e.g. topoisomerase II beta, RAD1, Ku autoantigen). Several encouraging results were also obtained in models of pancreatic carcinoma. Nawrocki et al. showed that bortezomib alone as well as in combination with docetaxel reduces tumour growth of orthotopic human pancreatic tumour xenografts [48, 49]. This significant tumour reduction can be attributed to an inhibited proliferation, increased apoptosis and reduced microvessel density. These data indicate that bortezomib inhibits growth of pancreatic tumours via direct effects on tumour cells and indirect effects on the tumour microenvironment. Furthermore, these results are supported by similar findings achieved in a xenotransplant model with human pancreatic carcinoma cells using the combination of the proteasome inhibitor MG132 and etoposide [50].

17.5.3.2 Inhibition of NF-κB Activation by Interfering with the IKK Complex

Thalidomide

Thalidomide was originally developed in the 1950s as a sedative and anti-nausea drug but was rapidly withdrawn due to teratogenicity. Meanwhile, thalidomide and its derivates (Actimid, Revlimid) have been proved to possess potent anti-tumour activity which is mainly based on the abrogation of NF-κB activation by inhibition of the IKKβ activity. Thalidomide has been shown to increase chemosensitivity in tumour models with melanoma [51] and glioblastoma [52]. Most intensively, the anti-tumour activity of thalidomide was investigated in experimental settings of multiple myeloma. Beside its potent anti-angiogenic activity (which is partially also mediated by NF-κB inhibition), thalidomide has shown several other anti-tumour activities in multiple myeloma cells: direct induction of apoptosis, growth arrest and inhibition of cytokine and growth factor secretion [53–55]. Mitsiades et al. could show that thalidomide treatment of multiple myeloma cell lines and cells from multiple myeloma patients increases sensitivity to apoptosis induced by Fas, Trail or dexamethasone [56]. Furthermore, thalidomide-treated cells exhibit a clearly reduced NF-κB activity, reduced expression of cIAP2 and FLICE inhibitory protein (FLIP) as well as an increased activation of caspase 8. Marriott et al. evaluated the anti-tumour activity of thalidomide and certain analogues in the treatment of different solid tumour cell lines (colorectal, pancreatic, prostate) in vitro and in vivo [57]. The thalidomide analogue phosphodiesterase type IV inhibitor effectively reduces tumour cell viability, thereby leading to inhibition of tumour growth in vivo. This effect appears to be mediated by decreased expression levels of bcl-2 and an increased induction of caspase 3.

CHS-828

CHS-828 (N-(6-(4-chlorophenoxy)hexyl)-N′-cyano-N″-4-pyridylguanidine) belongs to a new group of anti-tumoural substances, the pyridyl cyanoguanidines, and inhibits NF-κB activation by blocking IKK activity [58]. Hjarnaa et al. demonstrated that CHS-828 exerts significant cytotoxic effects on human breast and lung cancer cell lines that were not seen on normal fibroblasts and endothelial cells [59]. In nude mice bearing human tumour xenografts, CHS-828 reduces growth of MCF-7 breast cancer tumours and leads to the regression of small-cell lung cancer tumours. Aleskog et al. observed significant cytotoxic activity of CHS-828 in haematological as

well as in solid tumour cells, although haematological tumour cells appear to be more responsive than those of solid tumours [60]. Furthermore, CHS-828 induces significant cytotoxic effects in myeloma cell lines in vitro and in vivo [61]. A recent publication of Johanson et al. demonstrated anti-tumoural activity of CHS-828 against different neuroendocrine tumours [62]. One study has reported on the treatment of U-937 GTB lymphoma cells with a combination of CHS-828 and etoposide [63]. Some promising synergistic cytotoxic effects could be observed, but the enhanced apoptosis induction by etoposide apparently depends on the duration of exposure to CHS-828. Thus, further studies evaluating the therapeutic efficacy of CHS-828 in combination with etoposide or other chemotherapeutic drugs seem to be warranted.

Sulphasalazine

Sulphasalazine is an anti-inflammatory drug that has been used for a long time in the treatment of inflammatory bowel disease or of rheumatoid arthritis. It is metabolically cleaved following oral administration to 5-amino-salicylic acid (5-Asa) and sulphapyridine. Its mode of action has been linked to the ability to inhibit IKK kinase activity and hence the activation of NF-κB [34, 64, 65]. Sulphasalazine has been shown to inhibit proliferation of human mammary carcinoma cells [66] and lymphoma cells [67] in vitro. Robe et al. observed growth inhibitory properties of sulphasalazine in human glioblastoma cells in vitro and in vivo [68]. In addition, sulphasalazine is able to efficiently induce apoptosis in glioblastoma cells as determined by DNA fragmentation and caspase cleavage. Thus, decreased proliferation and increased apoptosis account for significant remission of experimental U87 tumours in the brain of nude mice after sulphasalazine treatment. Arlt et al. intensively evaluated the NF-κB blocking activity of sulphasalazine in the sensitization of pancreatic carcinoma cells in vitro [13, 69, 70]. Pre-treatment of chemoresistant pancreatic carcinoma cells with sulphasalazine clearly increases the apoptotic response towards cytostatic drugs such as etoposide, doxorubicin, gemcitabine or 5-fluorouracil. Moreover, combined treatment of severe combined immunodeficiency (SCID) mice bearing human pancreatic tumour xenografts with sulphasalazine and either etoposide or gemcitabine significantly reduces tumour outgrowth [50]. Immunohistochemical analysis revealed that tumours of combined treated animals exhibit a significantly increased number of apoptotic cells, a markedly reduced number of proliferating tumour cells and a decreased microvessel density, effects similarly seen with combined treatment of cytostatic drugs and the proteasome inhibitors MG132 [50] or bortezomib [48, 49].

17.6 NF-κB Inhibitors in Clinical Trials

17.6.1 Bortezomib

The proteasome inhibitor bortezomib has already applied in a variety of clinical studies to improve the therapy of different malignancies, a fact which is reflected by 196 entries in Medline if searching for the terms "bortezomib" and "clinical trial". The vast majority of studies were conducted with patients suffering from multiple myeloma [71–73]. Richardson et al. had 669 patients in their study comparing the therapeutic efficacy of bortezomib with high-dose dexamethasone in terms of tumour response, progression time and time of survival [73]. All patients displayed an advanced relapsed tumour stage and had received one to three previous therapies before entering the study. Patients that were treated with bortezomib showed a higher tumour response rate compared to patients treated with dexamethasone. The combined complete and partial response rates were 38% for the bortezomib group and 18% for the dexamethasone group ($p < 0.001$). Moreover, median times to progression were 189 days for patients after bortezomib and 106 days for patients after dexamethasone treatment, respectively ($p < 0.001$). The 1-year survival rate was 80% for patients receiving bortezomib and 66% for patients taking dexamethasone ($p < 0.003$). Most studies indicate that bortezomib is well tolerated. However, the most common grade 3 and 4 toxicities of bortezomib were thrombocytopenia, lymphopenia and peripheral neuropathy, the latter also being reversible after dose reduction or discontinuation [74]. Furthermore, bortezomib showed remarkable single-agent activity

in patients with other lymphomas such as non-Hodgkin's lymphoma or mantle cell lymphomas [75, 76].

In contrast, monotherapy with bortezomib exhibited no or only minimal activity in various advanced solid tumours such as metastatic sarcomas [77], metastatic colorectal carcinoma [78], metastatic malignant melanoma [79] or metastatic neuroendocrine tumours [80]. Thus, for further studies of solid malignancies, the authors univocally recommend the use of bortezomib in combination with cytostatic drugs. Aghajanian et al. determined in a single-arm phase I study the maximal-tolerated dose and safety of a combination of bortezomib and carboplatin in recurrent ovarian cancer [81]. Besides assessment of the recommended dose of bortezomib, the overall response rate was 47%, including five partial and two complete responses, one of the latter occurring in a patient with platinum-resistant disease.

So far, only one study has been performed evaluating the therapeutic efficacy of bortezomib in the treatment of metastatic pancreatic carcinoma [82]. There were 44 patients enrolled for treatment with bortezomib alone and 43 patients for combined treatment with bortezomib and gemcitabine. Response rates were 0% and 10%, the median times to progression were 1.2 and 2.4 months and median survival times were 2.5 and 4.8 months, respectively. Thus, bortezomib alone or a combination of this drug with gemcitabine did not yield better results in the treatment of metastatic pancreatic carcinoma than expected for gemcitabine alone. It has to be critically noted that treatment with gemcitabine alone was not the subject of this study, thus it does not allow for proper direct comparison. Furthermore, patients progressing upon bortezomib monotherapy were allowed to receive bortezomib with gemcitabine, hence attenuating the assessable effect of the combined treatment. Further studies—in particular for the treatment of solid tumour diseases—should be aspire towards combinations of bortezomib and cytostatic drugs to better exploit the benefit of proteasome-dependent NF-κB inhibition for chemosensitization.

17.6.2 Thalidomide

The first study showing anti-tumour activity of thalidomide in the therapy of refractory multiple myeloma was published in 1999 by Singhal et al. [83]. Since then, several clinical studies have been undertaken to prove therapeutic efficacy of thalidomide in the treatment of haematological as well as of solid tumours. Many encouraging results were obtained in phase II and III studies with advanced multiple myeloma [84–87]. Rajkumar et al. enrolled 207 patients with newly diagnosed multiple myeloma in a phase III study comparing the combination of thalidomide and dexamethasone (103 patients) with dexamethasone alone (104 patients) [84]. The combined treatment showed a significant better response rate compared to dexamethasone monotherapy (63% versus 41%, $p < 0.002$). Albeit this convincing anti-tumour activity was achieved by additional thalidomide treatment, a significant higher incidence of grade 4 and 5 toxicities (e.g. peripheral neuropathy) was observed in patients receiving combined treatment compared to patients treated with dexamethasone alone (45% versus 21%, $p < 0.001$). However, Kyriakou et al. described a combination of cyclophosphamide and dexamethasone with low-dose thalidomide as a well-tolerated and effective therapeutic regimen for patients with relapsed or refractory multiple myeloma [87]. Therefore, efforts still have to be undertaken to optimally balance maximal anti-tumour activity with the adverse effects of thalidomide.

Up to now, monotherapy with thalidomide hardly induced any objective response rates against solid tumours, e.g. of advanced melanoma, renal cell, ovarian or breast cancer [88, 89]. This holds true for the combination of thalidomide and capecitabine in the therapy of patients with metastatic colorectal carcinoma who were refractory to previous therapies [90]. Interesting results were obtained in a phase II trial treating patients with metastatic neuroendocrine tumours with a combination of thalidomide and temozolomide [91]. In this single-arm study, 40% and 29% of 29 patients showed an objective biochemical and radiologic response, respectively. The median duration of response was 13.5 months, the 1-year survival rate was 79% and the 2-year survival rate was 61%.

Gordon et al. evaluated in a radomized placebo-controlled trial the efficacy of thalidomide in the attenuation of tumour cachexia in 50 patients with advanced pancreatic carcinoma [92]. Tumour cachexia, which mainly depends on NF-κB activity and the release of pro-inflammatory cytokines [93], represents a common problem in approximately 80% of patients with pancreatic carcinoma and is associated with a much worse clinical outcome. In this study, 33 patients (16 placebo, 17 thalidomide) were evaluated after 4 weeks of treatment showing that patients receiving thalidomide had gained weight by an average of 0.37 kg compared with a median weight loss of 2.21 kg in the placebo group ($p = 0.005$). Moreover, evaluation of 20 patients (8 placebo, 12 thalidomide) after 8 weeks revealed a median weight loss of 0.06 kg in patients treated with thalidomide compared with a loss of 3.62 kg in the control group ($p = 0.034$). In conclusion, thalidomide was well tolerated and effectively diminished weight loss in patients suffering from advanced pancreatic carcinoma. It would be of great value to further investigate its anti-tumour activity, particularly in combination with cytostatic drugs in the therapy of pancreatic carcinoma.

17.6.3 CSH-828

Until now, two phase I studies have been conducted evaluating the maximal tolerated dose (MTD), the recommended dose and the toxicity of CHS-828 [94, 95]. Sixteen and 27 patients, respectively, with different histologically proved solid malignancies were included in these studies. Most patients had already received previous treatments with chemotherapy, radiotherapy, surgery, and/or hormonal therapy. For further studies, Hovstadius et al. recommended a dose of 20 mg CHS-828 once daily for 5 days in cycles of 28-days duration [94]. Haematological toxicity (thrombocytopenia, lymphocytopenia) was generally mild. Other side-effects were most frequently nausea, vomiting, diarrhoea, fatigue and localized genital mucositis. No objective tumour responses could be noted, although seven patients showed stable disease after two courses of therapy. Ravaud et al. concluded that a dose of 420 mg of CHS-828 administered every 3 weeks is recommended for further studies, while 500 mg is the MTD [95]. Haematological toxicities such as anaemia and thrombocytopenia as well as gastrointestinal side-effects (pain, nausea, vomiting, diarrhoea) were frequent. In both studies there was a large variation in pharmacokinetics of CHS-828 both between and within patients. To overcome this problem, a series of improved pro-drugs of CHS-828 was synthesized. The best compound was EB1627 showing improved solubility and potent anti-tumour activity alone or in combination with cytostatic drugs in animal models [96]. For further studies it will be worthwhile to evaluate whether combinations of CHS-828 or of the improved pro-drug EB1627 with other drugs might be more potent in the treatment of solid tumour diseases.

17.6.4 Sulphasalazine

Since Sulphasalazine has already been used for decades to treat inflammatory diseases and its administration has been proved to be safe and well tolerable, this drug seems particularly qualified for NF-κB inhibition in cancer treatment. Currently, a prospective, double-blind, randomized phase 1–2 study is being conducted to prove the safety and efficacy of sulphasalazine for the therapy of advanced malignant gliomas [97]. The primary study objectives are the evaluation of the maximal daily oral dose of sulphasalazine and the assessment of any clinical and radiological tumour responses. Determination of overall and progression-free survival will be secondary objectives. Overall, 20 patients will be enrolled in the study.

Based on our own comprehensive experimental and preclinical investigations [13, 50, 69, 70, 98], the University Hospital Schleswig-Holstein Campus Kiel will launch a pilot study applying sulphasalzine as a chemosensitizer for the treatment of pancreatic carcinoma. Overall, 20 patients with an advanced inoperable pancreatic adenocarcinoma will be enclosed in a prospective, single-arm, multi-centre study to explore the compatibility and efficacy of the combination of sulphasalazine and gemcitabine.

17.7 Concluding Remarks

Since inflammation, a crucial risk factor for tumour development and progression, and chemoresistance both broadly depend on activation of NF-κB, this signalling molecule represents an attractive target for cancer prevention and therapy. However, NF-κB in immune cells is an important mediator and regulator of immune function so that its permanent inhibition might lead to severe immunosuppression. Thus, prolonged inhibition of NF-κB seems not to be applicable for tumour prevention. In contrast, suppression of NF-κB activity might be more useful in the therapy of already existing tumours, thus implying NF-κB inhibitor administration will be of shorter durations. Although it must be kept in mind that under certain circumstances NF-κB inhibition might also contribute to tumour progression, the most likely outcome of this interference in existing tumours will be impairment of the tumour microenvironment (e.g. by reducing tumour vascularization) and increased tumour cell apoptosis. Since, in particular, solid tumours apparently exhibit comprehensive protection from apoptosis induction, the mere inhibition of NF-κB appears to be insufficient for a pronounced anti-tumour effect. Thus, it is reasonable to use NF-κB inhibitors as chemosensitizing adjuvants in combination with cytostatic drugs.

Several preclinical and clinical studies have revealed that the transcription factor NF-κB is a promising molecular target that can be used for sensitization of a variety of tumours to chemotherapeutic drugs. NF-κB inhibitors, such as bortezomib, thalidomide or sulphasalazine, that have been already employed in clinical studies should be further evaluated in combination with cytostatic drugs, particularly in the therapy of profoundly chemoresistant tumours (e.g. pancreatic carcinoma). One focus of recent research is the design and development of more specific IKK inhibitors; they will enter clinical application within the next few years. These improved NF-κB inhibitors will presumably have fewer side-effects with respect to immunosuppression and are likely to be more potent in anti-cancer therapy.

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