

Modulation of gene transcription by natural products – a viable anticancer strategy?

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Abstract

Drug design based on the structure of specific enzymes playing a role in carcinogenesis, e.g. tyrosine kinases, has been successful at identifying novel effective anticancer drugs. In contrast, no success has been achieved in drug design attempts, in which transcription factors or DNA-transcription factor complexes involved in the pathogenesis of human neoplasms were targeted. This failure is probably due to the fact that the mechanism of transcription regulation is probably too complex and still too inadequately understood to be a suitable target for drug design. It seems plausible that the high selectivity of some human tumors to some DNA-interactive anticancer drugs, e.g. cisplatin, is related to an effect on the transcription of genes that are crucial for those tumors. In this article we propose that some natural products have evolutionarily evolved to exert highly specialized functions, including modulation of the transcriptional regulation of specific genes. We discuss in detail the marine natural product Yondelis (Trabectedin, ET-743) that is effective against some soft tissue sarcoma, possibly because it interferes with the aberrant transcription mechanism in these tumors. In addition we highlight the existing evidence that many different natural products are effective inhibitors of NF- κ B, a transcription factor that plays a crucial role in inflammation and cancer, indicating that some of these compounds might possess antitumor properties. We propose that large-scale characterization of natural products acting as potential modulators of gene transcription is a realistic and attractive approach to discover compounds therapeutically effective against neoplastic diseases characterized by specific aberrations of transcriptional regulation.

Introduction

There are many ways to define the crucial differences between cancer cells and their normal counterparts, from which they are derived. For several human tumors it is thought that the crucial carcinogenic event is the mutation of genes encoding proteins important in cell function. In most cases several mutations are necessary to transform a normal cell into a malignant one. The complex biological phenomena related to the abnormal behaviour of cancer cells, defined as the hallmarks of cancer [1] include acquisition of self-sufficiency in growth signals, insensitivity to those signals which inhibit the proliferation, inactivation of apoptosis pathways normally occurring in irreversibly DNA damaged cells, ability to replicate indefinitely, initiation of angiogenesis ensuring sufficient oxygen and nutrient supply to sustain tumour growth and attainment of ability to invade and metastasise other tissues. All these phenomena are essentially related to important alterations in the pattern of gene transcription and gene expression, that ultimately regulate the normal mechanisms of cell growth, differentiation, survival, interaction with other cell types and angiogenesis. This fact may explain why gene expression patterns obtained by microarray analysis represent more accurate tools in both the classification of tumors and the definition of patients' prognosis than hitherto used morphological and clinical approaches. A logical therapeutic implication of this concept is that, in principle, finding effective ways to modulate gene transcription should be an effective strategy to achieve redifferentiation and normalization of cancer cells, thus accomplishing therapeutic control of the neoplastic disease. In the present paper we summarize the evidence for the contention that certain naturally occurring agents can modulate transcriptional regulation, and we proffer the argument that such modulation might be a mechanism which may be profitably exploited in new anticancer drug development.

Consequence of alteration of transcriptional regulation for carcinogenesis

A growing body of data indicates that the deregulation of transcription factors can be crucial in the pathogenesis of different tumors. Many translocations that are typical of specific neoplastic disease like most leukemias and sarcomas involve chimeric proteins encoded by fusion genes that represent the pathogenetic initiating factors in the carcinogenesis process. These chimeric proteins, which can regulate gene transcription, consist of two proteins or protein fragments, which themselves can be transcription factors. Chimeric proteins often maintain the ability of the constituent transcription factors to bind to DNA and transactivate gene transcription, even though they elicit abnormal regulation. For some tumors the precise cascade of events from gene translocation to change of cell phenotype is only partially elucidated. In some cases the deregulation of a tyrosine kinase is oncogenic, as in the case of chronic myeloid leukaemia expressing the Bcr-Abl fusion protein. In other cases the key element is a transcription factor that works in an anomalous fashion [2]. In several sarcomas the deregulated transcription factor is the crucial cancer-initiating event ultimately leading to the malignant phenotype [3-8], implying that such a factor is a potential target for specific and effective therapies for these diseases. As an example for this scenario table 1 shows the main translocations in human soft tissue sarcoma, which are responsible for the formation of fusion proteins acting as deregulated transcription factors.

Can small molecules modulate transcription factor function?

Many attempts have been directed at modifying gene transcription regulation in tumor cells. It has been known for a long time that aza-cytidine or its more specific analogue

aza-deoxy-cytidine modify regulation of gene transcription by inhibiting DNA methylases [9]. A large body of data supports the notion that the differentiation-inducing and antileukemic effects of these compounds is related to a change of the methylation status of the promoters of several genes, that ultimately results in cell death/differentiation depending on the cellular model. This mechanism is the basis for the clinical use of these compounds in the therapy of some haematological malignancies [10, 11]. Another class of compounds that are under clinical investigation are the histone deacetylase (HDAC) inhibitors [12]. Convincing experimental evidence exists that the acetylation status of histones H3 and H4 and other nuclear proteins, including p53, affect the transcriptional regulation of cancer-related genes. Thus HDAC inhibitors may exert efficacy by modifying the altered pattern of gene expression of cancer cells. Both, inhibitors of DNA methylation and of histone deacetylases, have shown significant biological and therapeutic effects against some neoplasms [10-12]. Their use in therapy is usually rationalised by the fact that carcinogenesis is associated with epigenetic events, not because they alter the transcriptional regulation of specific genes. Yet modification of the methylation or acetylation status of DNA and chromatin is unlikely leading to a specific anti-cancer effect. Inhibition of DNA methylation and histone deacetylation probably modifies the expression of a large number of genes, possibly including some mechanistically related to carcinogenesis or tumor progression, but also some related to normal cell function. Such compounds are potentially interesting as they engage a mode of action different from that of conventional anticancer drugs. But as their mechanism is not cancer-specific, their therapeutic index is necessarily limited. Nevertheless preclinical data supports the contention that inhibitors of DNA methylase or histone deacetylase can enhance the activity of other anticancer drugs by modifying resistance

mechanisms. These findings provide the rationale for ongoing clinical trials aimed at evaluating DNA-methylase and HDAC inhibitors in combination with other antineoplastic drugs.

The effect of cytotoxicants on transcription factors

The precise mechanism of action of most antitumor drugs has not yet been fully elucidated. For example, the reason why cisplatin is selectively effective against testicular cancer remains unresolved. None of the explanations that have been proposed so far have been supported by convincing data. For example, it has been suggested that the deficiency of some components of the DNA repair machinery, like the proteins XPA, involved in nucleotide excision repair mechanisms, e.g. XPA, a characteristic feature of testicular cancer, is the biochemical basis for the selective cytotoxicity of cisplatin [13-15]. However, this hypothesis is inconsistent with the fact that cells deficient in XPA are also very susceptible to DNA-alkylating agents such as L-PAM and nitrogen mustard [16], whilst this does not appear to be the case for testicular cancer. One might argue that the real mechanism by which cisplatin exerts its cytotoxicity, highly selective for testicular cancer and reasonably selective for other human malignancies, e.g. ovarian cancer, is related to its ability to block the transcription of genes essential for the maintenance of these tumors. It is likely that DNA interacting drugs such as cisplatin, which forms DNA-intra and DNA-interstrand crosslinks, modify DNA structure such as to render some consensus sequences for the binding of transcription factors unrecognizable, thus leading to alterations of transcriptional regulation. There are reports indicating that high concentrations of cisplatin or other DNA interacting agents, e.g. doxorubicin, prevent the binding of transcription factors to specific consensus sequences *in vitro* [17, 18].

However the relevance of these findings has been questioned because the supporting data has solely been obtained *in vitro* using concentrations of drugs that are much higher than those present *in vivo* in the biophase after treatment with tolerable doses. Therefore the interference by this type of drug with transcription factor - DNA interactions, whilst biochemically intriguing, is probably not an antitumor mechanism of action *in vivo*. Likewise DNA methylating drugs such as temozolomide pose puzzling mechanistic dilemmas. Temozolomide, the best available chemotherapy for CNS tumors [19-21], crosses the blood brain barrier and upon conversion by spontaneous hydrolysis to monomethyl-triazeno imidazol carboxamide is thought to methylate DNA. Methylation of DNA at the O⁶ position of guanine is crucial for experimental and clinical drug activity. However patients suffering from glioblastoma, that do not express the DNA repair protein O⁶-alkylguanine DNA alkyltransferase (AGT) because of the methylation status of the promoter of the gene, have a much greater probability of response and survival after temozolomide treatment than glioma patients expressing AGT [22, 23]. This finding intimates that alkylation of O⁶-guanine is important for the action of temozolomide. But why should temozolomide-mediated methylation of guanine be so cytotoxic and render some tumors selectively sensitive to this drug? Many years ago we made the observation that replacing guanine with O⁶-methylguanine can modify the recognition of transcription factors [24]. Whether this might be a potential mechanism rendering some tumors selectively sensitive to methylating agents has not elucidated. But it is somewhat perplexing that a relatively “soft” DNA damaging agent producing only DNA methylation is much more effective against glioblastoma than compounds that cause much more severe DNA damage, i.e. DNA crosslinking agents. This finding

suggests that temozolomide alters some events regulating gene expression rather than generally blocks DNA function.

Whilst all of these observations do not allow firm conclusions to be made, they suggest tentatively that the mechanism of antitumor specificity of some cytotoxic anticancer drugs is not due to their ability to inhibit DNA synthesis, but that cell-specific regulatory mechanisms are involved, one of these possibly being the regulation of gene transcription.

Marine compounds as modulators of gene transcription

One of the most potently cytotoxic natural products ever tested in panels of murine tumors and human tumor xenografts is ET-743 (YondelisTM, Trabectedin).

This compound which is isolated from the sea squirt *Ecteinascidia turbinata*, a tunicate that grows on the mangrove roots throughout the Caribbean sea [25], is a tetrahydroisoquinoline alkaloid that binds to the minor groove of DNA and subsequently forms covalent adducts by reacting with N2 of guanine to its carbinolamine moiety [26, 27]. Experiments in cells *in vitro* [28] suggest that ET-743 at concentrations that are pharmacologically reasonable (i.e. in the nM range) can specifically affect gene transcription in a promoter-dependent fashion [29, 30]. We have initially focussed our studies on NF-Y, that activates the CCAAT element present in approximately 25% of genes, many of which are involved in the regulation of the cell cycle and differentiation. Using NIH-3T3 cells transfected with xenopus HSP-70 with a CCAAT box in the promoter that is activated by NF-Y we demonstrated that ET-743 was able to inhibit the heatshock induction of HSP-70 transcription [29]. Interestingly inhibition was obtained when cells were exposed to ET-743 at concentrations of 10-100 nM, whereas it was not observed using other

DNA binding drugs at much higher concentrations, typically 10 μ M. The data obtained with different promoters indicates that the effect was rather specific and certainly not due to a PolIII inhibition. Other studies involving run on experiments in isolated NIH-3T3 nuclei confirmed the effects of ET-743 on the transcription of endogenous genes. These studies do not support the notion that the effects of ET-743 were the consequence of a general inhibition of transcription, as only a fraction of genes was affected. Some of these genes, like MDR1, c-jun, H2B or H4, contain functionally important CCAAT-boxes. However some genes that were affected, like c-fos, did not contain CCAAT-boxes, indicating that the effects of ET-743 were not specific for NF-Y but that other transcription factors were inhibited. Further studies were performed to investigate the effect of ET-743 on the promoters of genes (cyclin E, E2F1, TK, DHFR, cyclin A, cdc2 and cyclin B2) that regulate the cell cycle by using NIH-3T3 fibroblasts stably transfected with reporter vectors, containing either the CAT or luciferase genes, together with the thyromycin resistance containing vector [31]. Cells synchronized in G0 were stimulated to grow by serum addition, and at several time points the transcriptional activity of these promoters were studied in untreated and ET-743 treated cells. ET-743 caused a strong inhibition of cyclin B2 already at 1 nM, which might explain the G2 blockade induced by the drug. As far as G1/S promoters TK and DHFR are concerned, they were clearly inhibited, whereas cyclin E was increased at 4 out of 5 time points. Other promoters were not significantly affected. These data corroborate the idea that ET-743 is not a general inhibitor of transcription, but that it acts preferentially on some promoters by inhibiting or inducing their activity [31]. The conclusion is also supported by gene profiling analyses performed in different cell systems by different laboratories that showed that ET-743 affects the expression of a relatively limited number of genes,

some of which are down regulated and some upregulated following treatment [32, 33].

The precise mechanism by which ET-743 modulates transcription has not been elucidated yet. One can exclude that the mechanism involves changes in histone acetylation status or methylation of the promoters. Furthermore the sequence specificity of DNA binding of ET-743 is very limited and does not justify the discrepant results obtained on different promoters. Considerable progress has been made in recent years in the field of transcriptional regulation, but there are still many aspects that are unknown. For example the recruitment and function of cofactors of transcription complexes or chromatin remodellers are still poorly understood, it is conceivable that ET-743 interferes with these processes. The structural changes induced by ET-743 in DNA promoter sequences are not necessarily the only mechanism and it has been proposed that the drug interacts both with DNA and protein complexes [34]. Noteworthy from a clinical standpoint is the fact that ET-743 is one of the few molecules that has shown antitumor activity in human sarcomas. As pointed out before (see table 2) for many sarcomas the most frequent pathogenic lesion seems to be the formation of a fusion gene encoding transcription factors that are no longer normally regulated. It is therefore possible that the reason why ET-743 has antitumor activity against sarcomas is related to its effects on transcription. To speculate further, the blockade by ET-743 of the transactivating activity of fusion gene products acting as anomalous transcription factors would constitute a highly selective mechanism of action.

Although we have no formal demonstration that the antitumor activity of ET 743 is related to its effects on transcriptional regulation, we can exclude that it acts by inhibiting DNA synthesis. In fact the activity of ET-743 was unrelated to the rate of

cell proliferation, and it was particularly high for cells in G1 or quiescent cells like monocytes and macrophages. Recently ET-743 was reported to have anti-inflammatory properties, a mechanistic feature that seems related to modulation of expression of some cytokines and chemokines by monocyte and tumor-associated macrophages, that may influence tumor growth and angiogenesis [35]. These results intimate the intriguing possibility that compounds such as ET-743 may exert antitumor activity at the transcriptional level by modulating host-mediated events, e.g. inflammation and angiogenesis. In this context it seems pertinent to mention that much research is ongoing to identify compounds which inhibit HIF1 alpha, a transcription factor that is induced by hypoxia and promotes angiogenesis by activating the transcription of angiogenic factors like VEGF.

NF-kB as a target of naturally occurring agents

Nuclear factor of kB, commonly referred to as NF-kB, has an important role in various physiological processes as well as in the development of many human diseases including immune-related diseases and cancer. For this reason it has attracted a lot of research interest during the last decade [36]. NF-kB is implicated in regulating many fundamental pathways including immune response, cell growth and survival. Its deregulation is often associated with various malignancies and can also lead to death through different mechanisms [37, 38]. NF-kB is not a single protein but consists of at least 5 different transcription factors belonging to the Rel family: RelA (p65), RelB, c-Rel, NF-kB1 (p50) and NF-kB2 (p52). All the members of the NF-kB family are characterized by the presence of a Rel homology domain (RHD), located at the N-terminus of the proteins, which is involved in interaction with the inhibitor IkB, dimerization, sequence-specific DNA binding and contains the nuclear localization

sequence. Even if they share structural similarities, the five NF- κ B family members, can be further classified in two different groups depending on the differences in their synthesis and the structure and function of their C-terminus. The first group consists of RelA, RelB and c-Rel which all possess the transcriptional activation domain and are synthesized as mature proteins. NF- κ B1 and NF- κ B2, on the other hand, are generated from large precursor proteins (p105 and p100 respectively), and their C-terminal region lacks the typical transactivation domain, but harbors various ankyrin repeats, which are the characteristic domains of I κ B. Therefore these two members of the family are repressed unless a selective activating proteolytic cleavage of their C-terminus occurs. The NF- κ B members associate to form homo- and hetero-dimers, which are related to specific responses to different stimuli and possess different effects on transcription due to the diverse characteristics of the various family members [39]. An additional level of regulation consists in the different pattern of expression of the members of the family, which is tissue- and cell-specific for most of them. Only p50 and RelA are ubiquitously expressed, thus constituting the most common inducible forms of active NF- κ B.

There are two different activation pathways for NF- κ B, the canonical and the non-canonical one (see figure 1). They differ in physiological role and in pattern of activation. In the canonical pathway various stimuli, including pro-inflammatory ones and DNA damage, activate IKK probably through different upstream mediators, which trigger the ubiquitination of IKK alpha and the subsequent phosphorylation loop of the IKK catalytic subunit. These events lead to the activation of the IKK complex; which is responsible for the phosphorylation of I κ B, leading to its polyubiquitination and consequent degradation through the 26S proteasome. This in turn enables NF- κ B to translocate to the nucleus and start its regulatory activity. This pathway depends

pivotaly on IKK and I κ B activation. Deregulation in one of these steps can lead to constitutive activation of NF- κ B, which can inhibit apoptosis in neoplastic cells as demonstrated by various studies using inhibitors of I κ B phosphorylation. The recently discovered non-canonical pathway, on the other hand, depends on the stabilization and consequent activation of NIK through different stimuli such as BAFF, LT β and CD40L. Activated NIK then directly recruits and activates IKK α into the p100 complex causing its polyubiquitination and subsequent activation via the 26S proteasome. The processing of the p100 complexes leads to the formation of p52/p52 homodimers, which form a complex with the nuclear coactivator Bcl3 to regulate gene expression, and p52/RelB heterodimers, which can directly regulate gene expression [39]. The fundamental role of NIK in this pathway and the necessity of its stabilization provide possible explanations for the reason why activation of the non-canonical pathway is delayed compared to the classical pathway, and why it can be inhibited by protein synthesis inhibitors and proteasome inhibitors [37] like bortezomib, a drug with demonstrated activity in human multiple myeloma [40, 41]. In most normal cells the constitutive level of p100 processing is nearly undetectable, while it has been shown to be relatively high in many malignancies, mostly myelomas and leukemias. Intriguingly p100 knockout mice do not seem to develop any notable malignancies. Recent studies also showed that inhibition of p100 processing by down-regulation of p100 expression causes a significant reduction in tumor cell proliferation, thus eliciting interest in the possible role of this new pathway in cancer therapy. Lately, much interest has been raised in natural products which can inhibit NF- κ B [38, 42, 43]. These substances (see Table 2) are now under intense scrutiny for their potential use in oncology as single agents or as adjuvants of commonly used chemotherapeutics. The success achieved with the proteasome inhibitor bortezomib in

the therapy of myeloma has increased the desire to identify new compounds directed to elements of the NF- κ B pathway. It has been proposed that the selectivity of bortezomib for myelomas is related to its ability to inhibit the degradation of IKK, thus inhibiting the nuclear translocation of NF- κ B.

Many of the naturally occurring NF κ B inhibitors (Table 2) are ingested with the diet, and thus might prove to be relatively safe and well tolerated. Potential safety is a promising aspect of this type of agents, some of which exert putative cancer chemopreventive properties in experimental model systems. Epidemiological evidence hints at the possibility that people consuming sufficiently large amounts of these substances with their diet have a reduced risk of developing malignancies, especially those of the gastro-intestinal tract [44-47]. Overall the ability of a large number of natural products to act on NF- κ B represents an attractive opportunity for the identification of novel drugs active in the prevention and/or therapy of malignancies. It needs to be emphasized that most available data on these natural products has been obtained *in vitro*, thus without consideration of their metabolism *in vivo*. This is an important point because most natural products listed in table 2, when administrated *in vivo*, undergo rapid biotransformation. In most cases these compounds undergo enzyme-catalyzed conjugation with glucuronic acid, activated sulphate or glutathione generating pharmacologically inactive metabolites. The possibility cannot be excluded that molecules may be identified that do not undergo phase II drug metabolism, or that molecules can be chemically altered so as to prevent rapid *in vivo* biotransformation. Certainly the semi-synthetic approach to obtain potent inhibitors that are sufficiently stable to reach the tumor target *in vivo* in sufficient amounts to exert biological effects is an attractive area of research in the development of novel compounds acting on NF- κ B.

Conclusions

In the last decade experimental and clinical oncologists have started to develop anticancer drugs that have been designed to inhibit specific targets relevant to cancer cell growth, survival and angiogenesis [48, 49]. Good results have been accomplished in developing compounds directed towards tyrosine kinases. These kinases act as receptors of growth factors or in signal transduction-pathways that are up-regulated in many different tumors. Most striking results have been obtained in CML with the use of Glivec, a tyrosine kinase inhibitor that blocks Bcr/Abl, the fusion gene product that is the pathogenetic lesion typical of the disease. In the field of tyrosine kinase inhibitors there are examples of effective compounds directed towards the Erb b family, including Erb b1 (EGFR) and Erb b2 and towards angiogenic factors such as VEGF. Although hitherto the success achieved in terms of increase of survival of cancer patients is much more modest than anticipated, further improvement may be obtained by identifying the initial pathogenetic lesions of tumors, if the paradigm of glivec is to be generalized to other tumors. New inhibitors of enzymes involved in signal pathways that are abnormally regulated in cancer make it possible to block these pathways, thus theoretically reversing the malignant phenotype. It may be argued that the limited success obtained with the use of specific targeted therapies for most human solid tumors is due to the fact that the relevant biochemical abnormalities that are the cause of the neoplastic transformation- expressed in the cancer stem cells- are as yet not known. Therefore treatments are addressed at genetic lesions that, whilst possibly biological relevant, are not the crucial ones. For many tumors including several leukemias and sarcomas robust biological knowledge exists on the initiating pathogenetic lesions, mostly related to specific translocations with the expression of a

fusion gene responsible for an abnormal regulation of transcription mechanisms. Why has there not been more success in targeting the anomalous fusion proteins responsible for the deregulated transcription ultimately leading to neoplastic transformation? We surmise that the lack of success in designing molecules acting as specific modulators of the transcription of specific genes is related to the complexity of the mechanisms involved in transcription, many of which have yet to be fully elucidated. Transcriptional regulation is in fact the result of a cascade of reactions involving the binding of transcription factors to their cognate cis-acting promoter elements, recruitment of an array of co-factors, chromatin remodelling and the activation of enzymatic activities. All these steps are only partially known and this renders the design of specific gene-transcription modulators particularly difficult. In general, our current ability to design molecules that modify protein-protein interactions in a desired fashion is much more limited than our expertise in inhibiting the catalytic site of tyrosine kinase enzymes, the structure of which is fully described. This difference may explain why the only effective modulators of transcriptional mechanisms of specific genes found thus far are natural products, molecules that have probably evolved evolutionarily to exert highly specialised functions, or derivatives of such molecules. This consideration highlights the importance of the identification of natural products as potential antitumor drugs acting on specific gene-promoters. The examples provided in this article intimate that the approach is realistic. We surmise that the following three approaches may be reasonable strategic avenues in the exploitation of the pharmacological potential of naturally occurring modulators of transcriptional regulation: 1) identification of novel agents by large screening programs, 2) further improvement of our understanding of their mechanism of action at the molecular level, 3) modification of their structure in order to improve their

pharmacokinetic features and thus improve antitumor activity. The increasing knowledge of specific alterations of transcription occurring in different human neoplasms renders this approach very attractive in the quest to discover novel effective anticancer drugs.

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Table 1. Chromosomal translocations and genes involved in soft tissue sarcoma

	Translocation	Genes
Ewing sarcoma/PNET	t(11;22)(q24;q12) t(21;22)(q22;q12) t(7;22)(p22;q12) t(2;22)(q33;q12) t(17;22)(q12;q12) inv(22)	EWSR1, FLI1 EWSR1, ERG EWSR1, ETV1 EWSR1, FEV EWSR1, E1AF EWSR1, ZSG
Desmoplastic small round cell tumor	t(11;22)(p13;q12)	EWSR1, WT1
Extraskeletal myxoid chondrosarcoma	t(9;22)(q22;q12) t(9;17)(q22;q11) t(9;15)(q22;q21)	EWSR1, CHN RBP56, CHN CHN, TCF12
Clear cell sarcoma	t(12;22)(q13;q12)	EWSR1, ATF1
Alveolar rhabdomyosarcoma	t(2;13)(q35;q14) t(1;13)(p36;q14)	PAX3, FKH3 PAX7, FKHR
Myxoid liposarcoma	t(12;16)(q13;p11) t(12;22)(q13;q12)	CHOP, TLS (FUS) EWSR1, CHOP
Synovial sarcoma	t(X;18)(p11;q11)	SSX1, SYT SSX2, SYT
Alveolar soft part sarcoma	t(X;17)(p11.2;q25)	ASPL, TFE3
Dermatofibrosarcoma protuberans (and giant cell fibroblastoma)	t(17;22)(q22;q13)	COL1A1, PDGFB
Low-grade fibromyxoid sarcoma	t(7;16)(q32-34;p11)	FUS, CREB3L2 FUS, CREB3L1
Angiomatoid fibrous histiocytoma	t(12;16)(q13;p11) t(12;22)(q13;q12)	FUS, ATF1 EWSR1, ATF1
Congenital fibrosarcoma (and mesoblastic nephroma)	t(12;15)(p13;q25)	ETV6, NTRK3
Endometrial stromal sarcoma	t(7;17)(p15;q21)	JAZF1, JJAZ1

Molecular Class	Compound Name
Lignans	Manassantins (+)-saucernetin (-)-saucerneol methyl ether
Diterpenes	Excisanin Kamebakaurin
Triterpenes	Avicin Oleandrin
Polyphenols	Resveratrol Epigallocatechin gallate Quercetin
Glucosinolates	Sulforaphane Phenethyl Isothiocyanate Indole-3-Carbinol

Table 2: Plant-derived natural NF- κ B inhibitors

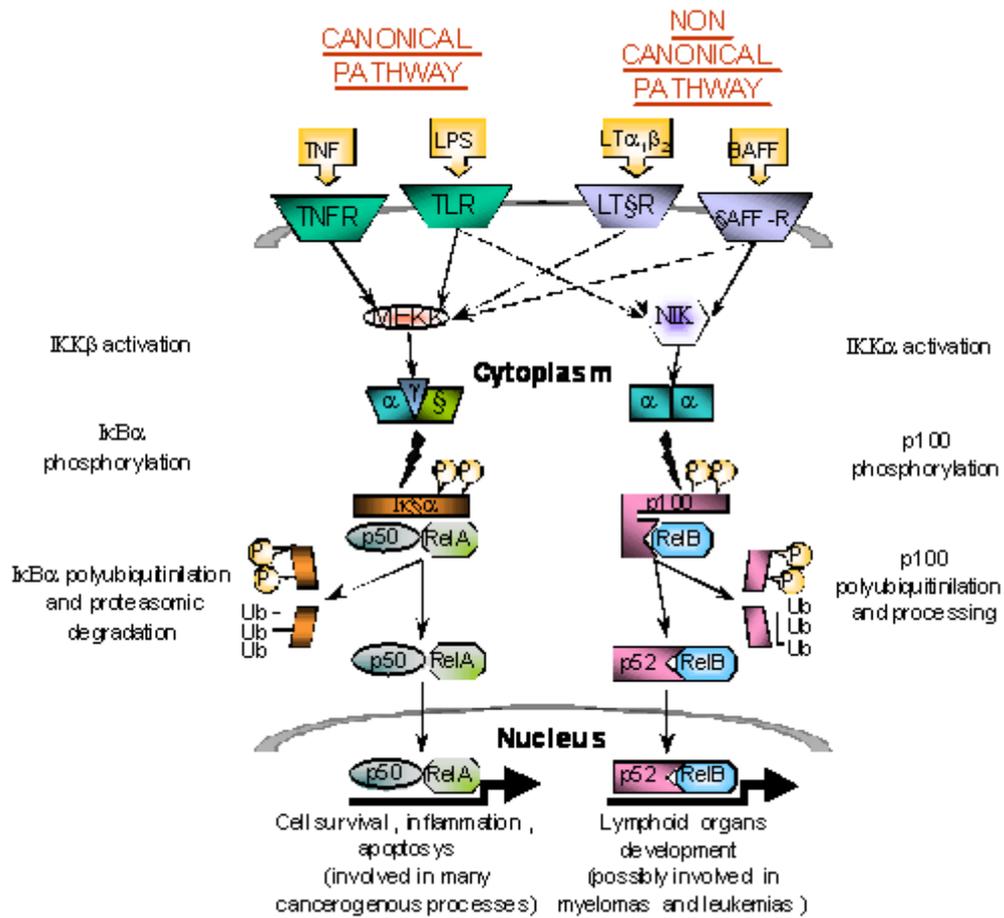


Figure 1. Activation of NF-κB by the classical pathway (left) and the alternative pathway (right). Signaling through TNFR, IL-1R, or Toll-like receptors (TLR) activates the classical NF-κB pathway involving predominantly the α and β subunits of the IKK complex. Nuclear translocation and DNA-binding of p50-RelA heterodimers is accomplished through IκBα phosphorylation and ubiquitin-dependent proteasomal degradation. Membrane-bound LTα1β2 heterodimers, CD40, and BAFF, on the other hand, activate via their respective receptors the kinases NIK and IKK. Phosphorylation of p100 results in the processing of the precursor to the p52 subunit and nuclear accumulation of p52-RelB heterodimers. There is significant cross talk since signaling through the LTαR, for instance, also results in the induction of RelA complexes and LPS can also trigger the processing of p100 to p52. It is likely that the two pathways activate distinct sets of genes. and BAFF, on the other hand, activate via their respective receptors the kinases NIK and IKK. Phosphorylation of p100 results in the processing of the precursor to the p52 subunit and nuclear accumulation of p52-RelB heterodimers. There is significant cross talk since signaling through the LTαR, for instance, also results in the induction of RelA complexes and LPS can also trigger the processing of p100 to p52. It is likely that the two pathways activate distinct sets of genes.