

Review

Transcription factor profiling unveils the oncogenes involved in the pathogenesis of cutaneous T cell lymphomas

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The finding in colon carcinoma that cancerogenesis is a sequence of activation of different oncogenes and inactivation of tumor suppressor genes has increased the efforts to identify the genes that are responsible for the progression of different kinds of cancer. Many activated oncogenes and inactivated tumor suppressor genes have been detected in cancer cells during the last decades, but for most cancers no network or sequence of oncogenes could be identified that could explain the progression of the disease and allow a molecular staging of the disease. Several strategies have been tried to find the genes that make cancer cells different from their normal counterparts, however, mostly only with little success. In this review article it will be reported how the strategy of transcription factor profiling helped to identify the genes that are responsible for the progression of two kinds of cutaneous lymphomas: Mycosis fungoides and the Sézary syndrome. By this way we were able to identify several agents that may be the prototypes of new drugs to fight these diseases.

Key words: Signal transduction, oncogenes, electrophoretic mobility shift, transcription factor ELISA, tyrosine kinase inhibitors, apoptosis inducers, skin cancer.

INTRODUCTION

Cutaneous T cell lymphomas

Cutaneous T cell lymphomas (CTCL) comprise a spectrum of lymphoproliferative disorders of the skin (Dummer et al., 2006). They affect mostly individuals over 50 years, but also younger people. The most frequent forms are mycosis fungoides (MF) and its leukemic counterpart, the Sézary syndrome (SS). MF evolves through several clinical stages (patch, plaque, and tumor stage) and leads ultimately to death often caused by rapidly growing and ulcerating tumours as well as by immune disorders. Lymph nodes are infested in later stages and infestation of inner organs is rare. The median survival time of MF patients is 8 - 20 years. Characteristics of the Sézary syndrome are generalised erythroderma, pruritus, dry skin, scaling especially at the palms, leukemic T cells in the blood and a shorter life expectancy (average 3 - 5 years). This is probably due to the circulating malignant T cells, which produce cytotoxic

T cells, suppressing interleukins like IL-10 in high amounts. At the moment there is no therapy to cure both forms of CTCL. In early stages CTCL is often misdiagnosed as eczema or psoriasis. Hyper- and hypopigmentation have also been found for MF and SS. Some clinical features of MF and SS can also occur in a transient stage of adult T-cell leukemia (ATL), which is caused by HTLV-I (Nagatani et al. 1994; Bunn and Foss 1996) and has a much more aggressive course. This has led to the erroneous assumption that HTLV may be involved in CTCL, which has been meanwhile disproved (Döbbeling, 2002 and references cited therein). The incidence of CTCL in the United States is 6.4/million/year (Criscione et al., 2007). The incidence in Afro-Americans has been found to be higher than in white Americans (9.0/million/year and 6.1/million/year respectively). However, it has to be kept in mind that MF and SS are clinically more difficult to discern from ATL on black skin. This has also been highlighted by the study of Fouchard

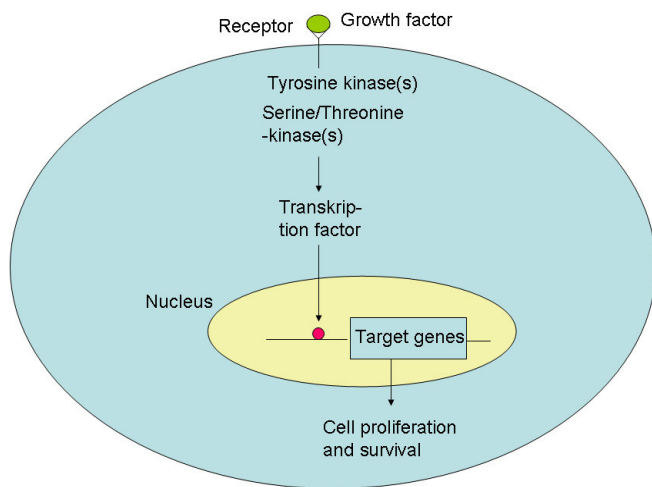


Figure 1. Schematic representation of the different steps of signal transduction and gene activation in the cell.

et al. (1998) in Mali. ATL cases may have been misdiagnosed as CTCL at times, when several immune histochemical and molecular biology methods have not yet been available (Bunn and Foss, 1996). These misdiagnoses may have also led to the conclusion that CTCL progresses more aggressively in Africans and Afro-Americans, since ATL progresses generally more aggressively than MF and SS.

MF patients in early stages are generally treated with glucocorticoids or more effectively with psoralen and subsequent UVA treatment (PUVA). PUVA therapy shows complete remission for 6 - 30 months in more than 80% of the patients. However all patients relapse. Advanced stages and Sézary syndrome are treated by PUVA plus interferon alpha or retinoids, extracorporeal photopheresis, total skin electron beam irradiation or chemotherapy (e.g. low dose methotrexate, chlorambucil plus prednisone, retinoids, interferon alpha, (Dummer et al., 2007). All these treatments lead to partial or total remission, but not to a final cure of the disease.

Molecular cancerogenesis

It has been shown for colon carcinoma that cancerogenesis is a multistep process which involves the mutation of several oncogenes and anti-oncogenes (Vogelstein et al., 1993). Many oncogenes are involved in signal transduction (e.g. src, ras, raf) and are often transcription factors (e.g. c-fos, c-jun, c-myc), which promote the entry of cells into a new replication cycle. Tumor suppressor genes are either regulators of the cell cycle preventing cells from uncontrolled growth (e.g. Rb, c-abl, p21, p16), or transcription factors, which enhance the transcription of these genes (e.g. p53). The expression of

proto-oncogenes is normally strictly regulated and does not lead to tumorigenesis. Only mutants of these genes which cannot be properly regulated gain a tumorigenic potential. Wild type oncogenes that function normally in contrast to their mutated forms are called proto-oncogenes.

There are different kinds of oncogenes. Many oncogenes code for molecules that are involved in signal transduction, these include growth factor receptors (e.g. EGF, PDGF receptors), tyrosine kinases (Jak, c-abl, c-src), serine/threonine kinases (raf, c-mos) and transcription factors (c-jun, c-fos, c-rel). The first step of a typical signal transduction pathway is that a growth factor binds to its receptor on the cell membrane. This leads to changes of the molecular structure of the receptor molecule (e.g. EGF, PDGF receptor), and the activation of a tyrosine kinase, which is part of the receptor molecule. In the case that the receptor does not possess a tyrosine kinase activity it can recruit directly or via adaptor molecules free tyrosine kinases to the receptor/ligand complex (e.g. Jak, c-abl, c-src). The activated tyrosine kinases then activate serine/threonine kinases, which activate transcription factors by phosphorylation. These activated transcription factors are now able to move to the nucleus and to bind to their target sequences on the DNA. Here they help to recruit co-factors of RNA polymerase to the promoter and to form an active transcription complex (Figure 1). This scheme has many variations e.g. some tyrosine kinases can directly activate transcription factors and sometimes a whole cascade of serine/threonine kinases (MAPKKK pathways) may be required for transcription factor activation.

In cancer mutations of signalling transducing molecules (receptors, tyrosine kinases, serine/threonine kinases, and transcription factors) can mimic a stage of permanent stimulation. The replacement of a tyrosine residue in tyrosine kinases, which often phosphorylate themselves, by an acidic amino acid (glutamic acid, aspartic acid) can lead to a permanently active tyrosine kinase molecule, since the negative charge of the acidic amino acid mimics the negative charge, which is normally introduced by the addition of a phosphate molecule to the tyrosine residue. Such a mutant would constitutively activate transcription factors, which in turn can activate genes that are necessary for proliferation and cell survival (e.g. bcl-2). Constitutive tyrosine kinase can also be achieved by the loss of a regulatory domain of the enzyme by deletion or missense mutants. The same types of mutations can also occur in serine/threonine kinases and transcription factors.

Every signalling pathway activates only a certain subsets of transcription factors. Thus it is possible to identify a disturbed signalling pathway by the determination of abnormal constitutive transcription activities in a cancer cell. There are inhibitors of tyrosine kinases, serine/

threonine kinases and transcription factors, which can serve as prototypes of future anti-cancer drugs. One example is Imatinib (Gleevec, STI157), which inhibits the tyrosine kinases c-abl and c-kit. It is effective against chronic myeloid leukemia (CML) (Deininger et al., 2005) and gastro-intestinal stromal tumors (GIST) (Schnadig and Balanke, 2006).

Detecting differences in gene expression in cancer and normal cells

The first strategy to detect genes that are involved in cancerogenesis was the construction of subtraction libraries. The basic principle was that the total m-RNA of one cell type was hybridized to the c-DNA library of another cell type and c-DNAs that did not hybridize and remained single stranded were cloned (Schweinfest et al., 1990). This method was quite successful when cells of different tissues were compared, but yielded often disappointing results, when cancer cell lines and the corresponding cells were compared.

The technically most sophisticated method to detect differences in gene expression in normal and cancer cells is expression profiling using DNA microarrays that are also known as gene chips. On one gene chip thousands of single stranded DNA oligonucleotides that represent sequences of genes are spotted and chemically linked to a chemical matrix (glass, plastic, silicon). The c-DNA libraries from two different cell types or cell lines that have been labelled with two different fluorescent dyes are hybridized to the chips. The binding of the differently labelled probes to a given spot results in an individual colour of each spot. From the different colours of the spots it can be deduced which gene more or less expressed in the analyzed cell lines. The exact determination of the wavelengths of the colours of the individual spots and the evaluation of the relative abundances of the investigated genes needs a lot of hard- and software for the evaluation. This method is therefore very expensive. The initial results were disappointing and little reproducible, since the comparison of the same cell lines with the gene chips of different suppliers yielded different results. This has meanwhile improved and the results that have recently been obtained for MF and SS cells (Mao et al., 2003; Mao et al., 2008; Vermeer et al., 2008; van Doorn et al., 2008) are reliable, since beside the identification of genes that expressed differently in MF and SS they could also reproduce findings that have been made before by the following method (Qin et al., 1999; Zhang et al., 2003; Zhang et al., 2007).

The strategy of transcription factor profiling

Transcription factors transmit the information of cell sig-

nalling to gene expression. They bind to specific DNA sequences around the coding sequence of the target gene and recruit RNA polymerases to the start point of gene transcription. Cancer cells have constitutive activities of transcription factors, which are inactive in normal cells. They receive activation signals from either mutated or otherwise deregulated kinases, which mimic signals of growth factors. Since only different sets of transcription factors are regulated by a given tyrosine or serine/threonine kinase, the identification of an abnormal transcription factor activity can be traced to a limited number of tyrosine and serine/threonine kinases, which then can be tested separately for their activities. Since each transcription factor regulates only a limited number of genes that favour the proliferation and survival of cancer cells, an aimed search for these target genes is possible.

METHODS USED IN TRANSCRIPTION FACTOR PROFILING

Preparation of nuclear extracts

To provide a transcription factor profile of a cancer cell line it is necessary to prepare protein extracts of the cell nucleus, which contains the transcription factors. A simple method to obtain such nuclear extracts has been developed by Gerber et al. (1992). The principle is that the cells are swollen in a hypotonic buffer and then lysed by a detergent. The nuclei are obtained by centrifugation and after the removal of the supernatant they are extracted by a high salt buffer. After the extraction the nuclei are removed and the supernatant containing the transcription factors can be stored at -80 or -190 °C.

Electrophoretic mobility shift assay (EMSA)

The EMSA or band shift is a quite old method, which has already been used for DNA binding proteins of bacteria. The adaptation of this assay to eukaryotic cells has helped a lot to identify eukaryotic transcription factors. The principle is that a radioactively or otherwise labelled double stranded DNA-oligonucleotide that contains the binding sequence of a certain transcription factor is incubated in the nuclear extract of a cell line of interest. After the binding reaction of transcription factor to the DNA has completed the mixture is loaded on non-denaturing polyacrylamide gel and the DNA and the transcription factor/DNA complexes are separated from the unbound DNA oligonucleotide by electrophoresis, since the transcription factor/DNA complexes migrate more slowly than the free DNA oligonucleotide. The presence of such complex confirms the presence of a transcription factor, but does not tell exactly which transcription factor it is, since nearly all transcription fac-

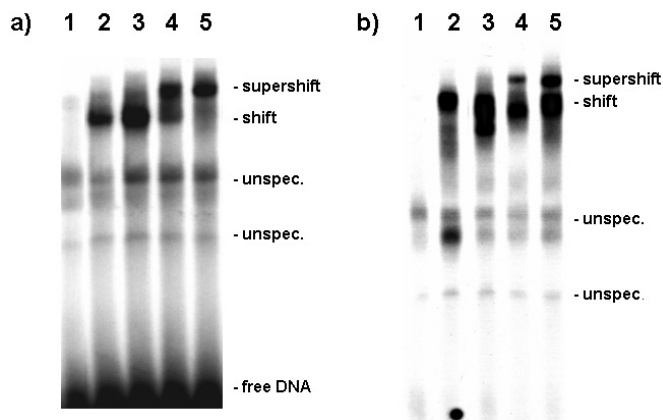


Figure 2. Sample of EMSA experiments with nuclear extracts of a) HUT78 (Sézary syndrome) and b) MyLa 2059 (Mycosis fungoides) cell lines and a DNA oligonucleotide containing the binding site of NFκB proteins. Lanes 1: negative control (untreated HeLa cervix carcinoma cells), lanes 2: positive control (phorbol ester treated HeLa cells). Lanes 3: untreated HUT78 (a) and MyLa 2059 (b) cells without antibodies, Lanes 4: untreated HUT78 (a) and MyLa 2059 (b) cells incubated with an antibody against NFκB p50, lanes 5: untreated HUT78 (a) and MyLa 2059 (b) cells incubated with an antibody against NFκB p65. Supershift: DNA/protein/antibody complex. Shift: DNA/protein complex. Unspec: unspecific DNA/protein complexes containing highly abundant proteins that bind sequence-independently to DNA. The gel with the MyLa 2059 cell nuclear extracts has been run longer for better resolution, so that the free unbound DNA has already passed through the gel.

tors belong to gene families, whose gene products often bind to the same DNA sequence. An exact identification of the transcription factor can be obtained, when a specific antibody against the supposed transcription factor is added to the nuclear extract and the DNA-oligonucleotide mixture. This leads to the formation of DNA/transcription factor/antibody complexes, which migrate on the polyacrylamide even more slowly produce a so called supershift (Figure 2). However, sometimes this trimeric complex is too unstable and cannot be seen on the gel.

Transcription factor ELISA (TF ELISA)

The difficulties with unstable DNA/transcription factor/antibody complexes can be overcome by the TF ELISA. The principle is that the DNA-oligonucleotide containing the DNA binding sequence of the transcription factor of interest is attached to the surface of a 96 well plate. Equal amounts of nuclear extracts of the cells of interest are added to the wells and the transcription factor of interest binds to its DNA motif. After the binding reaction has completed the remaining proteins are washed away. Then an antibody against the transcription factor is added. When the antibody has bound to its target a horseradish peroxidase (hrp) conjugated second antibody is added, which is directed against the constant region of

the first antibody. The addition of the hrp substrate to the well starts a colour reaction, which allows the measurement of the DNA-bound transcription factor by an ELISA reader. This method allows measuring many transcription factors and cell lines on plate at the same time.

Taken together these methods need less apparatuses, do not depend on sophisticated software, and are therefore more robust and less expensive.

IDENTIFICATION CONSTITUTIVE TRANSCRIPTION FACTOR ACTIVITIES IN MF AND SS BY TRANSCRIPTION FACTOR PROFILING

The NFκB transcription factor gene family

To profile mycosis fungoides and the Sézary syndrome, first the NFκB transcription factor family was analyzed. A screening of various B and T cell lymphomas (Frachiolla et al., 1993) had shown that 10 - 15% of CTCLs and the SS cell line HUT78 contain a chromosomal translocation of the NFκB2 (lyt-10) gene, which codes for the transcription factor NFκB2 (Neri et al., 1991; Schmid et al., 1991), also called p52. HUT 78 cells produce a truncated NFκB2 protein that is constitutively located in the nucleus (Zhang et al., 1994) since it lacks large parts of its regulatory region that retains it in the cytoplasm and thus prevents it to move into the nucleus and to bind to its target sequences on the DNA. This modified transcription factor therefore may act like other oncogenes (e.g. v-jun, v-Rel) as a permanently active transcription factor which disturbs normal gene expression.

NFκB2 belongs to the rel/dorsal/IκB superfamily of transcription factors, which consists of three groups (Baldwin 1996, Ghosh et al., 1998, Karin and Ben-Neriah 2000). Group I consists of proteins of about 65 kD (cRel, RelA, RelB) containing the rel-domain, which is necessary for DNA binding, activation of transcription and interaction with other members of the rel/dorsal/IκB-superfamily. Group II consists of the genes NFκB1 and NFκB2, which code for proteins of 105 and 100 kD respectively (p105 and p100). They contain a rel domain, an acidic region, and seven ankyrin repeats that tether these proteins to the cell membrane. The group II proteins p105 and p100 are processed by a protease into the transcriptionally active proteins p50 (NFκB1) and p52 (NFκB2) respectively, which lack the ankyrin repeats. The activity of the p100 processing protease depends on the activity of the IκB kinase alpha (IKK alpha) (Senftleben et al., 2001). Group III consists of the IκB proteins, which also contain seven ankyrin repeats and an acidic region, and resemble the carboxyl terminal parts of p105 and p100 proteins, which are cut off by a protease. The p50/p65 and p52/RelB proteins respectively form heterodimers that migrate into the nucleus, bind to NFκB sites on the DNA, and activate gene transcription. Group II proteins (p50 and p52) can

also form homodimers that also bind to the NFkB sites. They act generally as transcriptional repressors, but act sometimes as transcriptional activators, depending on the context of transcription factor binding sites in a given promoter or enhancer, and on the presence of cell specific co-factors (Kurland et al., 2001). The Ikb proteins (group III) bind to group I/II heterodimers and sequester them to the cytoplasm, blocking thus the activation of gene transcription by NFkB. When an NFkB activating stimulus is given Ikb proteins are phosphorylated by the Ikb kinase beta (IKK beta) complex and subsequently ubiquitinated and degraded (Zandi et al., 1997; Woronicz et al., 1997; Rothwarf et al., 1998). An exception amongst the Ikb proteins is Bcl-3. It forms p52/p52/Bcl-3 heterotrimers with p52 (Bours et al., 1993), which bind to NFkB sites and activate gene transcription. Bcl-3 has also been reported to activate transcription by the removal of p50/p50 homodimers from NFkB binding sites (Franzoso et al., 1992). The NFkB binding sites are not occupied by rel/dorsal/Ikb proteins in normal cells and binding of NFkB proteins occurs only after activation by a cellular signal e. g. by a cytokine (IL-1, TNF alpha) or a stress signal (hypoxia). However, for many cancer cells it has been found that they are state that resembles activated cells.

To profile mycosis fungoides and the Sézary syndrome nuclear extracts of mycosis fungoides and the Sézary syndrome cell lines were analyzed by electrophoretic mobility shift assays (EMSA) and transcription factor ELISA (TF-ELISA). Surprisingly all five members of the NFkB gene family: p50, p52 (group I), p65, RelB, and c-Rel (group II) were constitutively active in the tested MF and SS cell lines (Qin et al., 1997; Izban et al., 2000; Sors et al., 2006). Western blot analysis of the nuclear extracts confirmed that all NFkB proteins were of normal size with the exception of the p52 protein of the SS cell line HUT78, which carries an NFkB2 translocation. The Western blots also detected the Bcl-3 protein in the nuclear extracts of the tested MF and SS cell lines.

The immunohistochemical analysis of skin lesions of MF and SS patients with specific antibodies shows that p50 and Bcl-3 were present in the nuclei of malignant cells at the earliest stages of these diseases. The proteins p65, p52 and RelB were also found in earlier stage, but a simultaneous expression of p52 and RelB occurred only in the tumor stage (Döbbeling et al., 2007). The c-Rel protein was only found in some tumor stage samples. These findings indicate that the MF and SS cell lines mirror the tumor stage of MF and SS.

The STAT transcription factor gene family

Interleukin 7 (IL-7) and interleukin 15 (IL-15) have been found to be growth factors for MF and SS cells (Dalloul et al., 1992; Döbbeling et al., 1998a). Since interleukins sig-

nal through the Jak and Tyk tyrosine kinases, one can assume that the STAT (signal transducer and activator of transcription) transcription factors, which are directly phosphorylated and activated by Jak and Tyk tyrosine kinases, are involved in the pathogenesis of MF and SS. The test of the IL-7 and IL-15 independent MF and SS cell lines MyLa 2059 and HUT78 indeed detected constitutive DNA-binding of the STAT proteins 2, 3, 5 and 6 in the nuclear extracts of these cell lines. In the IL-7 and IL-15 dependent SS cell line however, the binding activities of these 4 STAT proteins were dependent on the presence of these two growth factors in the medium. All these four STAT proteins were of the expected size, indicating that no chromosomal translocations of these transcription factors has occurred. The fact that the MyLa 2059 and HUT78 have become IL-7 and IL-15 independent can therefore be explained by the assumption that a mutated or otherwise activated tyrosine kinase has become constitutively active in these cells (see below).

In the biopsies of MF and SS patients activated STAT5 was already detected in early stages, whereas STAT2, 3, and 6 become active only in the tumor stage (Qin et al., 2001a; Sommer et al., 2004). Recently it was reported that STAT3 is also already active in early stages (Witkiewicz et al., 2007). These activities may be triggered by tyrosine kinases that are IL-7 and IL15 independent or tyrosine kinases that are activated by IL-7 and IL-15 produced by skin keratinocytes and fibroblasts or the MF and SS cells themselves (Döbbeling et al., 1998a).

Constitutively active transcription factors of the jun/fos, myc/max and myb gene families

MF and SS cell lines and skin lesions were also tested for constitutively active members of the jun/fos, myc/max and myb gene families. For the jun/fos family no DNA binding activities could be found for the proteins c-Fos, Fra-1, Fra-2, and c-Jun, however constitutive DNA binding activities were reported for JunB (Mao et al., 2003) and JunD (Qin et al., 1999). Constitutive DNA binding was also observed for the myc/max and myb gene family members c-Myc, Max, and c-Myb. No binding was observed for N-myc and the mad proteins. The DNA-binding of JunD and c-myb was dependent on IL-7 and IL-15 in SeAx cells, whereas the binding of c-Myc/Max heterodimer was independent of IL-7 and IL-15 (Qin et al., 1999). In all three tested MF and SS cell lines two other IL-7 and IL-15 dependent DNA binding proteins were found, which bound like the c-Myc/Max heterodimer to the so called E-box DNA element, but did not react with c-Myc and Max antibodies. These complexes may contain the protein USF-1 and USF-2, since it has been reported that these proteins bind to the same recognition sequence as c-Myc/Max heterodimers. The c-Myb protein was detected in the MF and SS cell lines and skin biop-

sies. It could already be detected in early stage skin lesions (Qin et al., 2001).

Determination of the involved signalling pathways

Several substances are known to inhibit NFkB, STAT and Fos/Jun DNA-binding. Sodium salicylate is a potent inhibitor of the IKK beta (inhibitor of kappa B kinase beta) kinase. This kinase phosphorylates inhibitor of kappa B (IkB) proteins at a serine residue and targets it for proteolytic degradation. The destruction of the IkbS allows the NFkB molecules to enter the nucleus and to activate transcription. The addition of sodium Salicylate to MF and SS cell line led to a reduction of the constitutive NFkB binding and at longer incubation to the apoptosis of these cells. This experiment showed that constitutively active IKK beta activity is necessary for the maintenance of constitutive NFkB activities. The addition of the src-type tyrosine kinase inhibitor herbimycin A had the same effects, indicating that a src-type tyrosine kinase may be involved in NFkB and IKK beta regulation, since the prototype of these kinases, c-src, has been reported to phosphorylate IKK beta (Huang et al., 2003). An immunohistochemical screening of MF and tumors with specific antibodies showed that c-src and the related c-yes tyrosine kinase are expressed in these tumors. Interestingly c-src is also involved in the activation of STAT transcription factors. The expression of c-yes increased strongly in the tumor stage (Zhang et al., 2007). C-Src was already found in the early stages, however its activated (that is, phosphorylated) form was only found in the tumor stage. Therefore one can assume that interleukin-dependent tyrosine kinases are responsible for the phosphorylation of NFkB and STAT protein in the early stages, whereas the interleukin-independent c-Src kinase takes over this job in the tumor stage. The immunohistochemical stainings also revealed the expression of the tec-type tyrosine kinase bmx (Döbbeling et al., 2004), which has also been implicated in the activation of several STAT molecules (Saharinen et al., 1997). Sodium salicylate and herbimycin A could therefore be prototypes for novel anti-cancer drugs against MF and SS. In contrast to these two agents the NFkB and jun/fos antagonist dexamethasone had only a transient effect on the IL-7 and IL-15 dependent SS cell line SeAx. PP1, an inhibitor of the T cell receptor associated tyrosine kinases lck and fyn inhibited the constitutive NFkB activities, but had no proliferation inhibiting or cell killing effects, indicating that sodium salicylate and herbimycin A may have more targets than PP1 (Döbbeling et al., 1998b).

The IKK complex has also been reported to have an influence on the cell cycle. IKK alpha has been reported to suppress the cell cycle regulator p27 by the activation of p52/RelB heterodimers that activate the *skp2* gene. The Skp2 protein targets p27 for ubiquitin-dependent

degradation (Schneider et al., 2006). It is capable to inactivate RB by hyperphosphorylation (Li et al., 2003) and thus to increase cell cycle progression and proliferation. Since RB is hyperphosphorylated in MF and SS cell lines and skin lesions (Zhang et al., 2007), this could be another explanation of this finding, besides the already described inhibition of the p16 tumor suppressor gene (Zhang et al., 2007), which controls the phosphorylation of RB by cyclin dependent kinases.

VALIDATION OF THERAPEUTIC TARGETS BASED ON TRANSCRIPTION FACTOR PROFILING

Cell death inhibiting (anti-apoptotic) genes are targets of NFkB and STAT proteins

The anti-apoptotic *bcl-2*, *bcl-xL* and *mcl-1* genes have been reported to be regulated by NFkB and STAT proteins (Chen et al., 2000; Lord et al., 2000). Indeed the inhibition of NFkB and STAT factors by sodium salicylate and herbimycin A was paralleled with a reduced expression of these three genes (Döbbeling et al., 1998b). The inhibition of *bcl-2*, *bcl-xL* and *mcl-1* therefore seems to shift the equilibrium of the expression of pro- and anti-apoptotic genes to the side of the pro-apoptotic genes (Nielsen et al., 1999, Zhang et al., 2002). No effect on the two agents on the expression on the pro-apoptotic *bad* and *bax* genes was detected.

Inhibitors of *bcl-2* gene expression inhibitors are also inhibitors of NFkB and STAT in MF and SS cells

It has been reported (Shen et al., 1997) that arsenic trioxide (As_2O_3) that is used against AML (acute myeloid leukemia) (Soignet et al., 1998) is an inhibitor of *bcl-2*. Since MF and SS cells express *bcl-2*, As_2O_3 was tested for its ability to inhibit *bcl-2* expression in these cells. Indeed non-toxic concentrations of As_2O_3 (1 – 2 μM) suppresses *bcl-2* expression and causes apoptosis of MF (MyLa 2059) and SS cells (HUT78, SeAx) in cell culture. The suppression of *bcl-2* was paralleled by a repression of the DNA binding of NFkB and STAT protein. Thus the suppression of NFkB of STAT transcription factors may be the reason for *bcl-2* repression. The expression of the anti-apoptotic *bcl-xL* and *mcl-1* genes was also suppressed by the addition of As_2O_3 , whereas the expression of the pro-apoptotic *bax* and *bad* genes remained nearly unchanged (Tun Kyi et al., 2008).

Since As_2O_3 is a known poison it may be not well accepted by patients. Since arsenic and antimony have very similar chemical properties, we looked for antimony compounds that have already been used for treatment of other diseases. Finally we chose potassium antimonyl tartrate (PAT), which has been used for the treatment

against schistosoma and leishmania (Davis et al., 1975; Roberts et al., 1995) and has been reported to kill cells *in vitro* by oxidative stress (Lecureur et al., 2002).

Corresponding experiments as with As₂O₃ showed that PAT had the same effects as As₂O₃ (Qin et al., 1998). It killed the MF and SS cell lines *in vitro*, suppressed the DNA binding of NFκB of STAT transcription factors and suppressed the expression of the bcl-2, bcl-xL and mcl-1. The expression of the pro-apoptotic bax and bad genes remained also unchanged. The effects occurred at concentrations of 5 - 20 μM, depending on the cell line and were also observed in melanoma cell lines (Döbbeling et al., 1998c). These concentrations were 40 - 160 times lower than the LD₅₀.

Both agents were tested for their effects *in vivo*. For this purpose immune deficient athymic nude mice were used, which were injected with MyLa 2059 cells. Tumors appeared after 2 - 6 weeks. The minimal concentrations to reach total remissions were 0.5 - 1 mM As₂O₃ and 1 - 2 mM PAT injected directly into the tumor. Since these high concentrations were applied locally, they had no toxic side effects. This result indicated that MF tumor cells are much more resistant against anti-cancer agents, when they can grow as a tumor. This result explains why intravenous application of As₂O₃ in a 1 - 10 μM range did not affect skin tumors of Sézary syndrome patients although the leukemic cells in the blood were killed (Michel et al. 2003).

SUMMARY

Numerous proto-oncogenes that are involved in the pathogenesis of Mycosis fungoides and Sézary syndrome have been identified: c-rel, c-myb, c-myc, junB, junD, bcl-2, bcl-3, c-src, c-yes, bmi-1 and ras. Activation of c-myb, bcl-2, and bcl-3 occurs already in early stages of these diseases and is accompanied by constitutive activities of the transcription factors p50, p65, and STAT5. The other oncogenes and transcription factors described in this article are activated in the later stages.

The transcription factor profiling of Mycosis fungoides and the Sézary syndrome identified the NFκB and STAT factors and their signal transduction pathways as targets for new drugs for the treatment of these diseases. Prototypes of these drugs are the IKK beta inhibitor sodium salicylate and the src-type tyrosine kinase inhibitor Herbimycin A. Both reagents inhibit the signalling through the NFκB and STAT pathways and cause cell death by the inhibition of cell survival genes.

As₂O₃ and PAT, which have been initially described as cell survival (anti-apoptotic) gene inhibitors had the same effects on NFκB and STAT activities and cell survival gene expression as sodium salicylate and herbimycin A. They may therefore also act on the same kinases as salicylate and herbimycin A. The effects of As₂O₃ and

PAT could also be observed in a mouse model for MF (Tun Kyi et al., 2008; Tun Kyi et al., unpublished results). Experiments *in vitro* have also shown that As₂O₃ and PAT can also kill other cancer cell types indicating that these two agents may also be effective against other types of cancer.

From the data from MF and SS one can conclude that the following strategy may be successful for the search of new anti-cancer agent:

1. Identify aberrant constitutive transcription factor activities in cancer cells.
2. Determine the involved signal transduction pathways and transcription factor targets.
3. Identify known inhibitors of these pathways and targets.
4. Test these inhibitors *in vitro* and *in vivo*.
5. Look for or develop derivatives of these inhibitors that are more effective and less toxic.

Transcription factor profiling is also a useful tool to investigate the pathogenesis of cancers.

It could be shown that the different NFκB and STAT transcription factors are activated at different stages of the diseases. This allows determining how the diseases have progressed and which treatment may be the most suited one for the individual patient.

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