MYC in Oncogenesis and as a Target for Cancer Therapies

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MYC proteins (c-MYC, MYCN, and MYCL) regulate processes involved in many if not all aspects of cell fate. Therefore, it is not surprising that the MYC genes are deregulated in several human neoplasias as a result from genetic and epigenetic alterations. The near “omnipotency” together with the many levels of regulation makes MYC
an attractive target for tumor intervention therapy. Here, we summarize some of the current understanding of MYC function and provide an overview of different cancer forms with MYC deregulation. We also describe available treatments and highlight novel approaches in the pursuit for MYC-targeting therapies. These efforts, at different stages of development, constitute a promising platform for novel, more specific treatments with fewer side effects. If successful a MYC-targeting therapy has the potential for tailored treatment of a large number of different tumors. © 2010 Elsevier Inc.

I. C-MYC, MYCN, AND MYCL: THREE VERSIONS OF A MULTIFUNCTIONAL PROTEIN

The MYC gene was originally identified in avian retroviruses as the oncogene responsible for inducing myelocytomatosis in birds (Sheiness and Bishop, 1979). The cellular homologue, c-MYC, was found to be evolutionarily conserved (Vennström et al., 1982). Later, MYCN and MYCL were found amplified in neuroblastoma and in small cell lung cancer (SCLC), respectively (Henriksson and Luscher, 1996). These genes share the same general topography with the main open reading frame retained within the second and third exons. c-MYC is one of the most widely studied proto-oncogenes and it is localized to chromosome 8q24.21, a region that is translocated in Burkitt’s lymphoma (BL) (Dalla-Favera et al., 1982). The MYC genes encode short-lived nuclear phosphoproteins with a half-life of 20–30 min that are subsequently ubiquitinated for proteasomal degradation (Gregory and Hann, 2000). Human c-MYC encodes two major isoforms p67 (MYC-1) and p64 (MYC-2), with different expression patterns and biologically distinct functions (Hann et al., 1994). Transcription of MYC-1 is initiated at a cryptic start codon at the end of exon 1, whereas the more abundant MYC-2 protein is transcribed from an ATG start codon in exon 2, yielding a 439-residue protein. MYC is a basic Helix–Loop–Helix Leucine Zipper (bHLHZip) protein that heterodimerizes with the small bHLHZip protein Max resulting in dimers with DNA-binding ability at CACGTG and similar E-box sequences. The basic region (b) promotes sequence-specific DNA binding; the HLHZip confers protein–protein interaction while the Zip domain functions in cooperation with the HLH to stabilize protein–protein interactions and to establish dimerization specificity. Studies of the c-MAX protein revealed that the bHLHZip region conferring Max heterodimerization and specific DNA binding to the E-box is critical for all known MYC functions (Conzen et al., 2000; Luscher and Larsson, 1999). MYC is a multifunctional protein with the ability to regulate activities as distinct as cell cycle, growth and metabolism, differentiation, apoptosis, transformation, genomic instability, and angiogenesis (Fig. 1) (Meyer and Penn, 2008; Oster et al., 2002). It is believed that the majority of these functions are
exerted through gene regulation, which is supported by the findings that MYC interacts with proteins essential for transcriptional regulation, for example, transformation/transcription domain-associated protein (TRRAP) and histone acetyltransferases (HATs). These interactions occur through an evolutionarily conserved region called MYC Box 2 (MB2) within the transcriptional activation domain (TAD) in the N-terminus of the protein (Fig. 2) (Henriksson and Luscher, 1996; Meyer and Penn, 2008; Oster et al., 2002; Vita and Henriksson, 2006).

In addition to MB2 spanning residues 128–143 of the protein, the N-terminal TAD harbors another conserved region, MB1, encompassing residue 47–62 (Fig. 2). Several important functions have been ascribed to both MB1 and 2. As an example, MB1 encompasses the residues found to be required for MYC activity and breakdown (Henriksson et al., 1993; Oster et al., 2002) whereas MB2 is essential for cell transformation (Conzen et al.,

**Fig. 1  Different levels of MYC regulation and outcomes.** MYC activity can be regulated at the levels of [1] transcription, [2] translation, [3] ubiquitination and proteasomal degradation, [4] dimerization with Max, [5] dimerization with proteins other than Max (designated “X”), [6] DNA binding and target gene transcription, and [7] miRNA transcription. See text for details. Outcomes of MYC activation range from angiogenesis, proliferation, cell cycle, differentiation, cell growth, and metabolism, to apoptosis and if deregulated, transformation. MYC, c-MYC, MYCN, or MYCL protein; Ub, ubiquitin; miRNA, microRNA.
Fig. 2 Members of the two parallel networks with the Mlx protein in the center. The three MYC proteins are shown with MYC Boxes (MB) and bHLHZip domains. Dimerization partners are indicated by the dotted lines, connecting the bHLHZip regions of the respective proteins. Size and important structures of the proteins in the two networks are indicated. Mxd1 and -4 and the Mnt protein from the MYC/Max/Mxd network heterodimerize with both Max and Mlx, and the two Mondo proteins (A and B) belong to the Mlx network. NLS, nuclear localization signal; SID, Sin3-interacting domain.
More recently, two additional MYC boxes, MB3 (residues 188–199) and MB4 (304–324) were identified within the central region of the protein (reviewed in Meyer and Penn, 2008). MYC box 3 was found to play a role in cellular transformation (Herbst et al., 2004, 2005). Finally, MB4 overlaps with the nuclear localization signal (NLS)-1 and is required for MYC-induced focus formation in immortalized Rat1a cells, but not for cotransformation of primary rat embryo fibroblasts by activated Ras and MYC (Cowling et al., 2006). All four MYC boxes are conserved between species and are present in both c-MYC and MYCN whereas the more distantly related family member MYCL lacks MB3 (Ponzielli et al., 2005). In addition to NLS1 (320–332) within MB4, there is also an NLS2 (364–374). However, only NLS1 confers complete nuclear localization while NLS2 provides only a partial nuclear targeting, probably because it overlaps with the basic DNA-binding region. c-MYC and MYCN contain both NLS domains while MYCL harbors only NLS2 (Henriksson and Luscher, 1996).

A. Expression Patterns of the MYC Family Genes

Mouse models have revealed that c-MYC, MYCN as well as max are essential for survival, thus placing the network in a central position in the regulation of cell growth and homeostasis (Henriksson and Luscher, 1996). During early embryogenesis, there is some redundancy between c-MYC and MYCN since c-MYC^−/− and MYCN^−/− embryos survive until day 9–10, and day 11, respectively (Davis et al., 1993; Stanton et al., 1992). Such compensatory mechanisms are proposed to be possible only until MYC expression becomes more tissue-restricted during organogenesis. Expression of c-MYC is generally high during early embryonic development where it is required for embryonic stem (ES) cell pluripotency and reprogramming in addition to proliferation (Cartwright et al., 2005; Takahashi and Yamanaka, 2006). In differentiated adult tissues, however, the expression is low or undetectable consistent with the virtual absence of cell proliferation. In contrast to the almost ubiquitous expression of c-MYC, MYCN and MYCL expression levels are more restricted with respect to tissue and developmental stage (reviewed in Oster et al., 2002; Ponzielli et al., 2005). MYCN expression is very high early in embryogenesis in several tissues and declines dramatically during later development, generally coinciding with differentiation (Strieder and Lutz, 2002). The expression pattern of MYCL resembles that of MYCN but is even more restricted (Hatton et al., 1996; Zimmerman et al., 1986). After birth, MYCL is mainly expressed in the central nervous system, nasal epithelium, kidney, and lung. Neither MYCN nor MYCL expression correlates well with proliferation, further supporting
the notion that their expression is characterizing the undifferentiated state rather than promoting cell growth and division (reviewed in Henriksson and Luscher, 1996; Oster et al., 2002; Ponzielli et al., 2005).

B. Several Levels of Regulation

In resting cells, MYC mRNA and protein are virtually undetectable but the MYC levels increase rapidly after serum stimulation followed by a relatively slow decline initiated before the onset of S phase (Henriksson and Luscher, 1996). Protein synthesis is not required for the rapid and transient MYC induction during the G0/G1 transition. However, in contrast to many of the early response genes, MYC levels are maintained at a constant intermediate level in continuously proliferating cells.

In normal cells, MYC expression and activity is regulated at multiple levels through transcriptional, posttranscriptional, translational, and posttranslational mechanisms (Fig. 1). On the transcriptional level, MYC is regulated by signal transduction pathways that are activated both during normal development and in cancer. The most important include Sonic hedgehog, Wnt, Notch, receptor tyrosine kinase signaling, and transforming growth factor (TGF)-β (see below). At the posttranslational level MYC protein expression can be controlled through sequential and reversible phosphorylation at two highly conserved sites, threonine 58 (T58) and serine 62 (S62), located in the amino-terminal TAD of the protein (Henriksson et al., 1993 and reviewed in Hann, 2006). Phosphorylation of MYC at T58 and S62 regulates protein turnover through ubiquitination and 26 S proteasomal degradation (Bahram et al., 2000; Salghetti et al., 1999; Yeh et al., 2004). Phosphorylation at S62 increases MYC stability whereas T58 phosphorylation stimulates ubiquitination and degradation by the SCFβTrCP complex (Welcker et al., 2004; Yada et al., 2004; Yeh et al., 2004). Point mutation of MYC at either T58 or S62 has been reported in BLs as well as other lymphomas resulting in increased MYC protein stability (Bahram et al., 2000; Salghetti et al., 1999). However, point mutations have not been reported in solid tumors, despite the fact that several of these tumors exhibit stabilized MYC protein (Schulein and Eilers, 2009). Hence, other mechanisms are believed to be responsible for the increased stabilization of MYC proteins in some solid tumors.

It has been hypothesized that the glycogen synthase kinase (GSK)-3mediated phosphorylation of c-MYC at T58 and the subsequent dephosphorylation of S62 allows for binding of the ubiquitin ligase Fbw7 and recruitment of the SCFβTrCP complex to direct MYC ubiquitination and proteasomal degradation (reviewed in Dai et al., 2006). The Fbw7 tumor suppressor
protein is lost in many carcinomas, most notably that of colon (Rajagopalan et al., 2004). In contrast, the deubiquitinating enzyme USP28 that antagonizes the function of Fbw7, resulting in MYC protein stabilization, is overexpressed in breast and colon carcinoma (Popov et al., 2007). USP28 was found to be required for MYC function by forming a ternary complex with MYC and Fbw7 in the nucleus thereby preventing proteasomal degradation. Axin1, a scaffold protein that facilitates the interaction of c-MYC with GSK-3β, protein phosphatase 2A (PP2A), and the prolyl-isomerase Pin1 resulting in increased c-MYC ubiquitination, is inactivated through mutation in several cancers with high MYC expression (Arnold et al., 2009; Salahshor and Woodgett, 2005). In addition, a PP2A antagonist preventing S62 dephosphorylation of MYC was identified and designated cancerous inhibitor of PP2A (CIP2A) (Junttila et al., 2007). CIP2A was established as an oncoprotein, providing MYC stabilization as part of its oncogenic repertoire. Furthermore, as CIP2A is overexpressed in head and neck squamous cell carcinoma (HNSCC) and in colon cancer, it has been suggested that targeting CIP2A may be a possible treatment opportunity (Junttila and Westermarck, 2008; Junttila et al., 2007). Recently a number of reports have linked GSK-3β, Pin1, and PP2A as critical components of MYC protein degradation as GSK-3β phosphorylates MYC at T58 whereas Pin1 and PP2A cooperate to dephosphorylate S62 (Fig. 3) (Schulein and Eilers, 2009).

In addition to Fbw7, two other ubiquitin ligases are involved in regulating MYC protein turnover and/or activity, namely the F-box protein Skp2 and Hect H9, containing a Hect domain (Adhikary et al., 2005; Kim et al., 2003; von der Lehr et al., 2003; and reviewed in Dai et al., 2006). While Skp2 confers transcriptional activation as well as degradation of c-MYC (Kim et al., 2003; von der Lehr et al., 2003), HectH9 seems to only promote its transcriptional activity (Adhikary et al., 2005).

High expression of MYCN is an important mediator of proliferation of neural precursor cells during the development of the central nervous system. In mice, these high MYCN levels were shown to be caused by the persistent activation of phosphatidylinositol-3-kinase (PI3K)/Akt signal transduction by insulin or insulin-like growth factor (IGF) signaling which result in the phosphorylation of GSK-3β (Fig. 3) (Knoepfler and Kenney, 2006). Other proteins regulating the PI3K/Akt pathway, such as Ras, are frequently altered in human cancer, resulting in inhibition of GSK-3β activity and subsequently stabilization of MYC proteins through loss of T58 phosphorylation (Cully et al., 2006; Sears et al., 2000). In addition, a number of recent reports have shown that inhibiting key proteins in the PI3K/Akt signal transduction pathway results in the activation of GSK-3β with subsequent destabilization of MYC proteins (Chesler et al., 2006; Johnsen et al., 2008; Meyer et al., 2007; Mulholland et al., 2006). GSK-3β is also a key protein in
**MYC destabilized**

- LRPS/6
- Frizzled
- RTK
- Axin
- GSK-3
- β-catenin

**MYC stabilized**

- LRPS/6
- Frizzled
- RTK
- Axin
- GSK-3
- β-catenin

- PI3K
- AKT
- S473
- T308

**Ubiquitin-dependent degradation of MYC**

**Gene expression**
- c-MYC
- MYCN

**Induction of MYC target genes**
of the canonical Wnt/β-catenin signaling pathway by regulating the activity and nuclear translocation of β-catenin. In the absence of Wnt/Wingless ligand activation, β-catenin is sequestered in the cytoplasm by a multiprotein complex consisting of the adenomatous polyposis coli (APC) protein, Axin1, Axin2/Conductin, Casein kinase 1, and GSK-3β (Fig. 3). In this state, β-catenin is phosphorylated at amino-terminal serine and threonine residues by GSK-3β which targets it for ubiquitination and proteolytic degradation (Fodde and Brabletz, 2007). Activation of Wnt signaling by binding of Wnt ligands to a Frizzled receptor inhibits the formation of the multiprotein complex and GSK-3β-mediated phosphorylation of β-catenin resulting in an accumulation of hypophosphorylated β-catenin in the cytosol. Stabilized hypophosphorylated β-catenin eventually translocates to the nucleus where it interacts with members of the T cell factor/Lymphoid enhancer factor (Tcf/Lef) family of transcription factors, leading to increased transcription of a broad range of genes, including MYC (Fig. 3) (He et al., 1998). Hence, agents that directly target GSK-3β or key proteins in Wnt or PI3K/Akt signaling may have effects on MYC expression both through inhibition of MYC transcription and effects on MYC protein stability (Baryawno et al., 2010; Johnsen et al., 2008; Meyer et al., 2007; Chesler et al., 2006; Mulholland et al., 2006). Despite the high MYC protein turnover rate, it has been reported that the highly unstable pool of the protein coexists with a metabolically stable pool within the cell (Tworkowski et al., 2002). The difference in protein stability between these two pools is not due to cellular localization since they were both found within the nuclear compartment. In other cases, however, nuclear MYC protein has been detected predominantly in the cytoplasm. It has been suggested that hyperphosphorylation of MYC is one of the possible reasons for its redistribution to the cytoplasm (Oster et al., 2002). Furthermore, it has been proposed that the transcription factor Miz-1, which is repressed by MYC, also in some circumstances regulates MYC activity by controlling its nuclear import (Peukert et al., 1997).
II. NETWORKING IS KEY WITH MAX ACTING AS THE SPIDER IN THE WEB

As previously mentioned, the MYC dimerization partner Max, identified in 1991, was found to be an essential heterodimerization partner for all known c-MYC functions (Blackwood and Eisenman, 1991; Shen-Li et al., 2000). It has an important role in embryonic development as mice lacking max die at day 5–6 of gestation (Gilladogga et al., 1992; Shen-Li et al., 2000). Max is highly conserved in vertebrate evolution and, with a half-life longer than 14 h, is constitutively expressed in a number of different cell types. The two major splice variants encode Max p21 and Max p22, which both form homodimers as well as heterodimers with other network members (Fig. 2). The homodimers possess lower affinity to DNA, seem less discriminating compared to Max heterodimer complexes and their DNA-binding properties may be negatively affected by phosphorylation (Banerjee et al., 2006; Bousset et al., 1993; Brownlie et al., 1997). However, it is not clear whether the Max/Max homodimer has a function in vivo. In addition to the bHLHZip, Max contains an acidic region and a C-terminal NLS (Fig. 2) (reviewed in Henriksson and Luscher, 1996). Forced overexpression of max results in reduced growth (Zhang et al., 1997) and induced differentiation (Canelles et al., 1997).

In addition to the MYC proto-oncoproteins, the MYC/Max/Mxd network includes potential tumor suppressors (Mxd1–4 and Mnt) and Mga (Fig. 2). Whereas the Mxd proteins are mainly expressed in differentiated cells or tissues Mnt is expressed both in proliferating and differentiated cells and could function as a master regulator of MYC activity (reviewed in Wahlstrom and Henriksson, 2007). Some of the Mxd proteins interact with Mlx, another bHLHZip protein, suggested to be the center of a parallel network including the transcriptional activators Mondo A and B, proposed to regulate energy metabolism (reviewed in Billin and Ayer, 2006). However, the exact function of this parallel network is still poorly understood (Billin and Ayer, 2006). Thus the MYC network is in fact part of an intricate protein web. The crystal structure of the MYC/Max and the Mxd/Max complexes at the E-box revealed that there were marked structural differences in the dimerization patterns of the two heterodimers (Nair and Burley, 2003). While the MYC/Max heterodimers formed bivalent heterotetramers upon DNA binding, Mxd/Max complexes did not. The large size of the MYC/Max heterotetramers explains their ability to reach E-boxes spaced far apart and thereby to upregulate expression of such genes. Downstream effects are partially mediated through modifications of the chromatin structure to control DNA accessibility (Oster et al., 2002; Ponzielli et al., 2005).
A. Protein Interaction and Downstream Effects

Potential MYC-interacting proteins include the pRb-like p107 protein, the coactivator TRRAP, the multifunctional nucleolar protein, nucleophosmin (NPM), the tumor suppressor alternative reading frame (ARF), the transcriptional repressors TFII-I and Miz-1, as well as proteins responsible for MYC ubiquitination and proteasomal degradation discussed above (for review, see Dai et al., 2006; Li and Hann, 2009; Oster et al., 2002).

The interaction with p107 seems to involve a regulatory loop where the growth-inhibitory effects of p107 are counteracted by MYC while p107 significantly inhibits MYC-mediated transcriptional activation. However, p107 is unable to repress mutant c-MYC in BL (Gu et al., 1994), possibly due to N-terminal alterations, preventing Cdk1-Cyclin A-mediated c-MYC phosphorylation. Such c-MYC mutations may be one way for the protein to escape regulation and contribute to oncogenesis (Hoang et al., 1995). MYC also associates with HATs to acetylate histones and enable a transcription-permissive state of the chromatin at its target. The histone acetylation complexes are mainly recruited through the coactivator TRRAP, which was found to be essential for the transforming activity of MYC (McMahon et al., 1998, 2000). Observed interactions with other HAT complexes and TRRAP-independent molecules with chromatin remodeling capacity raised the possibility that MYC could also recruit other complexes for controlling target gene transcription (Oster et al., 2002). ARF has been shown to interact directly with c-MYC, leading to inhibition of its transforming activity while enhancing its apoptotic activity, independently of p53 (Li and Hann, 2009). NPM is another interactor that regulates c-MYC target gene expression by stimulating cell proliferation and transformation (reviewed in Li and Hann, 2009). c-MYC has also recently been suggested to have a function in chromatin dynamics since it was shown that its carboxy terminal region directly binds to the core subunit of the ATP-dependent chromatin remodeling complex: switching defective/sucrose nonfermenting (SWI/SNF), known as INI1/hSNF5. This hypothesis is further strengthened by the demonstrated interactions with other molecules with implications in chromatin remodeling, such as the ATPases/helicases TIP48/49 and the actin-related protein BAF53 (reviewed in Stojanova and Penn, 2009).

A number of MYC target genes have been described and documented in the MYC Target Gene Database: http://www.myc-cancer-gene.org/site/mycTargetDB.asp. Some examples are genes encoding Ornithine decarboxylase (Odc), p53, Carbamoylphosphate dihydroorotase (Cad), hTERT, and cell-cycle regulators such as Cdk4, Cyclin D, and E2Fs (for reviews, see Henriksson and Luscher, 1996; Meyer and Penn, 2008; Oster et al., 2002; Ponzielli et al., 2005). The Odc enzyme controls polyamine biosynthesis and
is essential for progression into S phase. MYC-mediated Odc upregulation may contribute to the oncogenic phenotype since Odc overexpression in mouse fibroblasts results in transformation (Moshier et al., 1993). The tumor suppressor protein p53, as previously described, is important in the cellular response to DNA damage with the ability to induce cell-cycle arrest or apoptosis (Sherr and Weber, 2000). MYC may activate p53 as a safeguard mechanism to prevent transformation by inducing apoptosis in MYC-overexpressing cells (Hermeking and Eick, 1994). Control of the G1/S transition is partially conferred by MYC through transcriptional induction of the Cad enzyme, required for de novo pyrimidine synthesis (Boyd and Farnham, 1997; Bush et al., 1998; Miltenberger et al., 1995). The catalytic subunit of telomerase (hTERT) also harbors E-box-elements to which MYC/Max as well as Mxd1/Max complexes have been shown to bind (Oh et al., 2000; Wang et al., 1998; Xu et al., 2001). Indeed, activation or repression of MYC has been shown to alter hTERT activity both in normal and tumor cells (Grand et al., 2002; Oh et al., 1999; Wu et al., 1999). Sustained MYC-induced telomerase activity has been reported in breast cancer, SCLC, and medulloblastoma (Geng et al., 2003; Li et al., 2002; Shalaby et al., 2010). The gene encoding the cell cycle regulator Cdk4 was found to have four conserved MYC binding sites in its promoter and is a direct MYC target gene (Hermeking et al., 2000). The incomplete cdk4 induction observed in MYC deficient Rat1 cells presented a link between cell cycle regulation and the oncogenic effect of MYC. The MYC-driven cell-cycle regulation was shown to be deficient in MYC-overexpressing breast cancer cells where CDK4 was no longer responsive to MYC (Pawar et al., 2004). Transcriptional regulation of D-type Cyclins may be another way through which the cell cycle progression is delayed in MYC null Rat1 cells. This hypothesis is strengthened by the fact that MYC upregulated transcription of Cyclins D1 and D2 (Bouchard et al., 1999; Perez-Roger et al., 1999), and also to some degree Cyclin D3 (Yu et al., 2005). Thus, it was suggested that upregulation of individual D-type Cyclins was sufficient to mediate the oncogenic effect of MYC. NBS1, encoding the Nijmegen breakage syndrome (Nbs)-1 kinase, a component of the MRN complex (Mre11/Rad51/Nbs1) has also been identified as a MYC target (Chiang et al., 2003). In a collaborative study, we recently showed that MYC-mediated control of transcriptional expression and nuclear translocation of Nbs1 is essential for regulating phosphorylation of the checkpoint response kinase ATM (Guerra et al., 2010). In addition to targeting other proteins, it has been suggested that the MYC gene itself harbors MYC-responsive elements and can regulate its own expression (Facchini et al., 1997). Taken together, these findings delineate a role for MYC in activating target gene transcription. MYC-mediated repression through interaction with TFII-I and Miz-1 is described below.
III. MYC-MEDIATED REPRESSION

Transcriptional repression by MYC is mainly mediated through protein-protein contacts, where MYC antagonizes the function of other transcriptional activators, without direct contact with the DNA (Kleine-Kohlbrecher et al., 2006). For instance, c-MYC-mediated inhibition of transcription can be conferred through interaction with TFII-I in the transcription machinery, binding at initiator elements (Roy et al., 1991). Together with observations that MYC-mediated repression by MYC-interacting zinc-finger protein-1 (Miz-1) also started from the initiator element (Seoane et al., 2002; Staller et al., 2001), it was originally believed that the initiator (Inr) element was a prerequisite for MYC-mediated transcriptional repression. In the case of Miz-1, a ternary complex with Max is required to mediate transcriptional repression of the Miz-1 target genes p21 and p15 (Herold et al., 2002; Seoane et al., 2002; Staller et al., 2001; Wu et al., 2003) as well as of Mxd4 (Kime and Wright, 2003). Physical interaction between MYC and Miz-1 is conferred by binding of Miz-1 to the HLH domain of MYC. This interaction appears to be MYC-specific as both c-MYC and MYCN bind to Miz-1 while neither Max, nor the HLH protein USF can bind (Peukert et al., 1997). It has been shown that MYC represses transcription of Miz-1 targets by displacing the Miz-1 coactivator p300 (Staller et al., 2001). This in turn enables MYC to recruit the Dnmt3a DNA methyltransferase corepressor to Miz-1, thus mediating repression by DNA methylation (Brenner et al., 2005).

MYC also controlled expression of c/EBP-α, one of the first MYC target genes shown to be repressed through an Inr element (Li et al., 1994). However, MYC also represses genes without Inr elements, such as p21, through interaction with the Sp1 transcription factor (Gartel et al., 2001). Furthermore, the MYC/Max complex was found to transrepress the p27 gene by directly binding to an Inr-like element at the promoter (Yang et al., 2001). Another mechanism for MYC-mediated repression of target genes occurs through recruitment of an mSin3/HDAC complex, when associated with MM-1 (Satou et al., 2001). Smad2 and NF-Y are two other interaction partners involved in MYC-mediated repression (Feng et al., 2002; Izumi et al., 2001). Interestingly, it appears that the conserved N-terminal MB2, important for MYC-mediated transactivation, is also essential for its repressive function (Conzen et al., 2000; Lee et al., 1997).

IV. INDUCTION OF APOPTOSIS

As mentioned above, MYC is a multifunctional protein and one of its important functions is the potentiation of apoptosis in response to cellular stress (reviewed in Nilsson and Cleveland, 2003). Cyclin A and Odc are two
potential mediators of MYC-induced apoptosis since Odc-blockage inhibits apoptosis in MYC-overexpressing cells and forced expression of Cyclin A is sufficient to induce apoptosis under low serum conditions (Hoang et al., 1994; Packham and Cleveland, 1994). Ectopic expression of Cyclin A could also restore apoptosis in c-MYC null cells treated with etoposide (Adachi et al., 2001). Induction of apoptosis by c-MYC has also been correlated with regulation of the Fas receptor and its ligand as well as proapoptotic Bax (Albihn et al., 2006; Fulda et al., 1998; Juin et al., 1999; Mitchell et al., 2000; Soucie et al., 2001). Bax appears to be essential for signaling c-MYC-induced apoptosis although there are few reports describing changes in Bax levels in response to c-MYC overexpression (Brunelle et al., 2004; Eischen et al., 2001; Juin et al., 2002; Mitchell et al., 2000). Instead the effect on Bax may be indirect by regulating upstream molecules such as Caspase 8, which is frequently inactivated in childhood neuroblastomas with amplified MYCN (Teitz et al., 2000). There is also the possibility of direct protein–protein interaction, since MYC under some circumstances can localize to the cytoplasm (Oster et al., 2002). The relation between MYC and the tumor suppressor protein p53 is complex. Even though p53 has been found to be important but not required for c-MYC-induced apoptosis, there are numerous tumor cell lines with deregulated c-MYC that carry p53 mutations or deletions (Gaidano et al., 1991; Wagner et al., 1994). In response to c-MYC activation and MYC/Ras-induced transformation, p53 is upregulated and stabilized to induce cell cycle arrest or, if the cell cycle blockade is overcome by c-MYC, apoptosis (Wagner et al., 1994). However, MYC-induced apoptosis may also be indirect by accumulation of reactive oxygen species (ROS) as a consequence of NF-κB inhibition (Pelengaris and Khan, 2003).

The many and diverse effects of c-MYC in promoting pathways as distinct as proliferation and apoptosis has brought forth the proposal of a model where activated MYC promotes apoptosis as the preferred physiological response. In case of excessive amounts of survival factors or mutations in the apoptotic pathway, the cellular MYC response would instead be uncontrolled proliferation. This model has been coined “the dual signal model” (Harrington et al., 1994; Hueber and Evan, 1998), and is supported by the observation that different regions of the c-MYC N-terminal domain can control distinct biological functions, including apoptosis (Chang et al., 2000; Conzen et al., 2000).

V. REGULATION OF STEMNESS

Analysis of transgenic mice with conditional expression of c-MYC or MYCN has shown that they are essential for normal developmental control of hematopoietic and neural stem cells, respectively (Knoepfler et al., 2002;
Wilson et al., 2004). MYCN has been shown to be required for normal neural stem cell function whereas c-MYC deficiency results in accumulation of defective hematopoietic stem cells (HSCs) due to niche-dependent differentiation defects (Baena et al., 2007; Wilson et al., 2004). It was recently shown that immature HSCs coexpress c-MYC and MYCN mRNA at similar levels and double knockout of c-MYC and MYCN results in pancytopenia and rapid lethality (Laurenti et al., 2008). Moreover, c-MYC is crucial for self-renewal and maintenance of pluripotency in murine ES cells. Murine ES cells can be maintained as a pluripotent, self-renewing population by leukemia inhibitory factor (LIF)/STAT3-dependent signaling which directly regulates the expression of c-MYC. Following LIF withdrawal, MYC mRNA levels collapse and MYC protein becomes phosphorylated on threonine 58 (T58), triggering its GSK-3β-dependent degradation. However, forced expression of stable MYC (T58A) renders self-renewal and maintenance of pluripotency independently of LIF (Cartwright et al., 2005).

Direct reprogramming of somatic cells provides an opportunity to generate patient- or disease-specific pluripotent stem cells. Murine and human somatic cells can be reprogrammed to pluripotency through generation of induced pluripotent stem cells (iPS) by retrovirus-mediated introduction of Oct3/4 (also known as Pou5f1), Sox2, Klf4, and c-MYC (Okita et al., 2007; Wernig et al., 2007). However, reactivation of the c-MYC retrovirus increases tumorigenicity in the chimeras and progeny mice, impeding clinical applications (Okita et al., 2007). Although a recent report shows that MYC may be redundant in the conversion of somatic cells to iPS cells (Nakagawa et al., 2008), there is still an implication for a novel stemness function of MYC that may be of importance for controlling tumor initiating cells.

Evidence suggests that MYC’s role in pluripotency is connected to its ability to regulate the cell-cycle machinery (Singh and Dalton, 2009). Elevated c-MYC levels accelerate the progression of cells through G1 phase by positively regulating cyclin-cdk activity (Amati et al., 1998). Conditional loss of MYCN in neural stem cells has been correlated with increased levels of the cdk inhibitors p18INK4c and p27KIP1 and decreased expression of cyclin D2 resulting in deregulation of the cell-cycle program (Knoepfler et al., 2002; Singh and Dalton, 2009). In addition, cell-cycle changes that occur during differentiation of pluripotent cells coincide with downregulation of MYC levels and the regulatory subunit of telomerase (TERT) in murine ES cells (Cartwright et al., 2005; Kim et al., 2008; White and Dalton, 2005). Finally, microRNAs (miRNAs) have been suggested to contribute to MYC’s role in pluripotency, either by coordinating the expression of cell-cycle molecules such as p21 and cyclin D2 in ES cells (Judson et al., 2009), or by repressing mRNAs involved in differentiation (reviewed in Dang, 2009).
VI. ONCOGENIC PROPERTIES

A major fraction of all human cancers display deregulated MYC activity (Nilsson and Cleveland, 2003; Ponzielli et al., 2005). Alterations include chromosomal translocations exemplified by the c-MYC-Immunoglobulin (Ig) fusion gene in BL (Hecht and Aster, 2000) and increased c-MYC expression due to gene amplification (Hogarty, 2003) as well as protein stabilization (Sears et al., 2000). Other oncogenic features are induction of genomic destabilization (Felsher and Bishop, 1999b; Mai et al., 1999), increased vascularization, and angiogenesis (Oster et al., 2002).

Oncogenes that frequently synergize with c-MYC in transformation include BCL-2, RAS, RAF, and c-ABL (reviewed in Oster et al., 2002; Pelengaris et al., 2002). c-Abl and Bcl-2 have been proposed to negatively regulate MYC-induced apoptosis. Consequently, a large proportion of tumors with deregulated c-MYC expression overexpress Bcl-2 (Cory and Adams, 2002). There are also reports on MYC regulation of Cyclin D1 and D2, both of which seem essential for MYC-driven proliferation, the gene encoding the latter being a direct MYC target, activated in response to growth factor stimulation (Bouchard et al., 2001). The nuclear zinc-finger protein encoded by BMI-1 synergizes with c-MYC in lymphomagenesis, possibly by negative regulation of p19ARF in the ARF–p53–MDM2 pathway (Jacobs et al., 1999). Prosurvival molecules that protect cells from c-MYC-induced apoptosis, such as the IGFs and platelet-derived growth factor (PDGF), may also facilitate transformation (Harrington et al., 1994). In addition, one mediator of the IGF-1-antiapoptotic effect, the bHLH family member Twist, promotes oncogenesis by inhibiting the apoptotic function of p19ARF (Dupont et al., 2001).

VII. NO TRANSFORMATION WITHOUT MYC?

Even though the generally accepted view is that cooperation of two oncoproteins such as MYC and ras are sufficient for cellular transformation (Land et al., 1983), this only holds true for murine cells, whereas additional events are required in human cells (Boehm et al., 2005; Hahn et al., 1999). The initially identified four events were expanded to six when the viral element (SV40 large T oncoprotein) was substituted for c-MYC. It was found that inactivation of tumor suppressor genes (p53, pRb, and PTEN) and limitless ability to replicate (hTERT activation), together with oncogene activation, were prerequisites for human cell transformation (Boehm et al., 2005). In fact, recent evidence has shown that the involvement of MYC may be necessary for oncogene-induced transformation (Soucek et al., 2008;
Zhuang et al., 2008). c-MYC overexpression was shown to partially overcome senescence induced by the oncoproteins B-Raf or N-Ras in a p53-independent manner, thereby contributing to the malignant phenotype in melanoma cells (Zhuang et al., 2008). In a different setting, inhibition of endogenous MYC was sufficient to trigger regression of Ras-induced lung adenocarcinomas in vivo, suggesting that MYC, even at its endogenous state is of vital importance for maintaining Ras-dependent tumors (Soucek et al., 2008). This newly identified importance of MYC, together with the finding that effects on normal regenerating tissues were well tolerated and completely reversible in the mouse, reinforces the prospect of targeting this nearly omnipotent oncogene as a feasible antitumor therapy. It was recently shown that the cooperation between MYC and ras in transformation of rodent cells required cdk2-induced phosphorylation of the MYC residue S62 (Hydbring et al., 2009). This in turn indicates that pharmacological inhibition of Cdk2 may be considered as an important cancer therapy for MYC-driven tumors.

VIII. MYC-ASSOCIATED CANCERS AND THEIR TREATMENT

The MYC family genes are deregulated by different mechanisms in several human neoplasias of different origin, including diffuse large B cell lymphoma, multiple myeloma, colon cancer, glioblastoma, melanoma, ovarian cancer, and prostate cancer (Nesbit et al., 1999; Vita and Henriksson, 2006). However, the extent of MYC involvement in these malignancies varies depending on the staging and the cancer form (Caccia et al., 1984; Nesbit et al., 1999; Pelengaris and Khan, 2003; Vita and Henriksson, 2006). Here, we focus on breast cancer, BL, lung cancer, medulloblastoma, neuroblastoma, and rhabdomyosarcoma (RMS); malignancies for which MYC has been shown to be important for development, progression, and/or patient risk-stratification. MYC status as well as current and future treatment approaches, some of which are already in clinical trials and others undergoing preclinical assessment, will be discussed (summarized in Table I).

A. Breast Cancer

Human breast carcinomas are heterogeneous, both in their pathology and molecular profiles and are the second most common cause of cancer deaths in the world. Molecular characterization of breast tumors have not revealed any common dominant pathway for the development or histological
<table>
<thead>
<tr>
<th>Cancer</th>
<th>MYC status</th>
<th>Current treatment</th>
<th>Treatments in clinical trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td><em>c</em>-MYC overexpression (45%)</td>
<td>Surgical resection, radiation, combinational chemotherapy, hormones, herceptin,</td>
<td><strong>Anti-HER2 therapy</strong> (pertuzumab, trastuzumab-DM1, KOS-953), aromatase inhibitors, tyrosine</td>
</tr>
<tr>
<td></td>
<td><em>c</em>-MYC amplification (9–48%)</td>
<td>lapatinib, antiangiogenic therapy</td>
<td>kinase inhibitors, mTOR inhibitors, endocrine therapy, stem cell transplantation, <strong>antisense</strong></td>
</tr>
<tr>
<td></td>
<td>MYCN overexpression (25%)</td>
<td></td>
<td><strong>therapy</strong>, imatinib+ vinorelbine, triptorelin, misteltoe</td>
</tr>
<tr>
<td>Burkitt’s</td>
<td><em>c</em>-MYC translocation (100%)</td>
<td>Short intensive multiagent chemotherapy, CNS prophylaxis, rituximab</td>
<td><strong>Different multiagent chemotherapy combinations</strong>+rituximab, stem cell transplantation</td>
</tr>
<tr>
<td>lymphoma</td>
<td><em>c</em>-MYC overexpression (91%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung cancer</td>
<td><em>c</em>-MYC amplification (20%)</td>
<td>Surgical resection, radiation, combinational chemotherapy, antiangiogenic therapy,</td>
<td><strong>Tyrosine kinase inhibitors and antibodies combined with chemotherapy, antiangiogenic therapy,</strong></td>
</tr>
<tr>
<td></td>
<td>MYCL amplification (13%)</td>
<td>EGFR-targeted therapies</td>
<td>mTOR inhibitors, EGFR inhibition (cetuximab), proteasome inhibitors, NSAID, zileuton</td>
</tr>
<tr>
<td></td>
<td>MYCN amplification (10%)</td>
<td></td>
<td>immunotherapy, cancer vaccines, talactoferrin, retinoids, misteltoe, green tea extract,</td>
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<td></td>
<td></td>
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<td>nanoparticles</td>
</tr>
<tr>
<td>Medulloblastoma</td>
<td><em>c</em>-MYC amplification (6%)</td>
<td>Surgical resection, radiation, combinational chemotherapy</td>
<td><strong>Temozolamide</strong>, irinotecan, immunotherapy, GDC-0449, erlotinib, dasatinib, mTOR inhibitors,</td>
</tr>
<tr>
<td></td>
<td>MYCN amplification (4%)</td>
<td></td>
<td>Met inhibitor, antiangiogenic therapy, HDAC inhibitors, Notch inhibitors, PARP inhibitor, nifurtimox</td>
</tr>
<tr>
<td>Disease</td>
<td>MYCN amplification (%)</td>
<td>Treatment</td>
<td></td>
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<tr>
<td>Neuroblastoma</td>
<td>25–30%</td>
<td>High-dose chemotherapy, surgical resection, myeloablative consolidation chemotherapy with autologous stem cell rescue, radiotherapy, 13-cis retinoic acid (RA), meta-iodobenzyl guanidine (MIBG)</td>
<td></td>
</tr>
<tr>
<td>Rhabdomyosarcoma</td>
<td>43–67%</td>
<td>Radiation, multiagent chemotherapy, surgical resection</td>
<td></td>
</tr>
</tbody>
</table>

Ch14.18 delivery+ IL-2 and GM-CSF, EBV-specific CTL, temozolamide, fenretinide, vorinostat+cis-RA, TNP-470, PF-02341066, ABT-751, MLN8237, CEP-701, SF1126, ZD6474, Proteasome inhibitors, nifurtimox, cixutumumab, R(+) XK469, OPT821+β-glucan

Treatments in bold italics are mentioned in the text.

*Studies not concluded. Source: www.cancer.gov/clinicaltrials/search/*
presentation with the result that breast tumors historically have been categorized into at least 18 different subtypes. More recently, gene expression profiling offered a way of classifying breast carcinomas into five different subtypes based on their mRNA profiles; luminal A, luminal B, Erbb2, basal, and normal-like (Sorlie et al., 2001). Both c-MYC (45%) and MYCN (25%) overexpression as well as c-MYC gene amplification (9–48%) have been described in breast carcinoma (Vita and Henriksson, 2006). Amplification of c-MYC is often correlated with a poor prognosis, in particular when it coincides with inactivation or mutation of the tumor suppressor gene BRCA1 (Chen and Olopade, 2008). Besides this, the correlation between MYC status and clinical outcome is less consistent (reviewed in Chen and Olopade, 2008). Activation of MYC in breast carcinomas has been correlated with the activation of Wnt, Notch, and TGF-β signaling (Chen et al., 2001; Klinakis et al., 2006; Ozaki et al., 2005; Stylianou et al., 2006). For example, nuclear localization of β-catenin was shown to be both strongly correlated to MYC expression and significantly correlated with reduced APC levels in primary breast cancer samples (Ozaki et al., 2005). MYC has also been shown to suppress DKK1 and SFRP1, two inhibitors of Wnt signaling thereby activating the Wnt pathway and promoting anchorage-independent growth of human epithelial mammary cells (Cowling et al., 2007). Recently, prolyl-isomerase (PIN) 1-dependent activation of Notch signaling was shown to activate MYC and transform normal epithelial breast cells (Klinakis et al., 2006; Rustighi et al., 2009). Finally Smads, a family of transcription factors that are regulated by TGF-β signaling and inhibit MYC expression, were shown to be decreased in breast cancer (Chen et al., 2001). The low expression of Smads significantly correlated with high tumor grade, larger tumor size, and hormone receptor negativity (Jeruss et al., 2003).

At the posttranslational level, MYC has been shown to be stabilized through inhibition of T58 phosphorylation by the activation of the estrogen pathway in ER-α-responsive breast carcinoma cells or elevated phospholipase D activity in ER-negative breast cancer cells (Rodrik et al., 2006). Detailed analyses of MYC amplification and elevated MYC expression in breast cancer samples have implicated MYC as a potential prognostic factor. However, more details on the expression levels and posttranslational modifications of MYC will be required in order to fully evaluate the importance of MYC expression in the initiation and progression of breast carcinomas.

B. Treatment of Breast Cancer

Different chemotherapy regimens, hormone therapy, and targeted therapy comprise the current standard (Engel and Kaklamani, 2007). Increasingly, treatment is becoming individualized and different prognostic and
diagnostic markers are tested for reliability in choosing the most suitable therapy (Bouchalova et al., 2009). Chemotherapy regimens include combinations of anthracyclines and taxanes, sometimes together with an antimetabolite such as 5-fluorouracil. The anthracyclines daunorubicin, doxorubicin, and epirubicin, all induce similar damage to cellular DNA and RNA (Rabbani et al., 2005), while the taxanes paclitaxel and docetaxel are antimicrotubule agents interfering with cell division (Abal et al., 2003). These treatments are sometimes combined with the CMF regimen which is a combination of the alkylating agent cyclophosphamide (C; or ifosfamide) with the antimetabolites methotrexate (M) and 5-fluorouracil (F) (Levine and Whelan, 2006). It has been hypothesized that c-MYC-amplified breast tumors are those that respond favorably to the CMF therapy (Bouchalova et al., 2009), supported by the fact that c-MYC transcription is suppressed in response to 5-fluorouracil treatment. In contrast, tumors where c-MYC was coactivated with E2F responded poorly to TFAC (paclitaxel, 5-fluorouracil, doxorubicin, and cyclophosphamide) therapy, but were predicted to be more sensitive to docetaxel containing regimens (Salter et al., 2008). Possibly, the sensitivity to docetaxel could be due to a decreased E2F activation.

Hormone therapy is used for treatment of hormone-receptor positive breast cancer patients. The hormonal therapeutics includes aromatase inhibitors, selective estrogen receptor modulators (SERMs), and estrogen receptor downregulators (ERDs) (Bush, 2007; Prat and Baselga, 2008). Aromatase inhibitors such as anastrozole, exemestane, and letrozole prevent the production of estrogen in postmenopausal women by blocking the activity of the enzyme aromatase that normally converts androgen into estrogen (Bush, 2007). It has been found that aromatase inhibitors are sometimes better than SERMs, such as tamoxifen, raloxifene, and toremifene in breast cancer treatment and prevention (Santen et al., 2003). However, the benefit with SERM treatment is that it can be used in women both before and after menopause. Similarly to SERMs, the ERD fulvestrant blocks the estrogen receptor (ER) and prevents estradiol from binding and eliciting downstream effects (Bush, 2007). It also reduces the number of ERs and interferes with the function of existing ERs. c-MYC appears to be a target of estrogen action, specifically mediating its effects in cell growth (Musgrove et al., 2008). Deregulated c-MYC was also shown to confer resistance to antiestrogen therapy in MCF7 cells by downregulating the expression of p21 (Mukherjee and Conrad, 2005). Consequently, breast tumors with deregulated c-MYC may not respond well to hormone therapy.

Targeted therapies include use of the monoclonal antibody herceptin (trastuzumab), blocking the effect of the growth factor protein Her-2. This treatment, combined with chemotherapy is useful in approximately 25% of women suffering from Her-2-positive breast cancer (Prat and Baselga, 2008). Likewise, the tyrosine kinase inhibitor lapatinib blocks the effect of
the Her-2 protein and other tumor-specific proteins. Lapatinib is used in combination with capecitabine for treatment of Her-2-positive breast cancer patients that no longer respond to treatment with herceptin. Another type of targeted therapy is the prevention of tumor growth by blocking angiogenesis (Sirohi and Smith, 2008). The monoclonal antibody avastin (bevacizumab), used in combination with paclitaxel, exerts its antiangiogenic effect by blocking vascular endothelial growth factor (VEGF) required to stimulate angiogenesis. Recent results suggest that c-MYC is important in estrogen-induced VEGF transcription (Dadiani et al., 2009), which would indicate that deregulated c-MYC would diminish the effect of avastin.

Other Her-2 targeting approaches are currently in clinical trials. The recombinant antibody pertuzumab prevents Her-2 dimerization, trastuzumab-DM1 combines the activity of trastuzumab with inhibition of tubulin polymerization, while KOS-953 is an Hsp90 inhibitor promoting ubiquitination and degradation of Her-2. Additional clinical trials exploit sentinel lymph node biopsy followed by surgery, high-dose chemotherapy with stem cell transplantation, and antisense therapy (reviewed in Prat and Baselga, 2008).

C. Burkitt’s Lymphoma (BL)

BL is a non-Hodgkin’s B cell lymphoma originally detected as an endemic form carrying a latent Epstein–Barr virus (EBV) infection. Later, sporadic and AIDS-associated BL, where the majority of tumors are negative for EBV, were described (Magrath, 1990). HIV infection and malaria are the most important risk factors for the development of BL through the induction of polyclonal B cell activation and hypergammaglobulinemia (Bornkamm, 2009). The common trait for BL is the development of tumors in extranodal sites in adolescents or young adults. The disease is classified as a distinct category of peripheral B cell lymphomas, and comprises a heterogenous group of highly aggressive B cell malignancies (reviewed in Hecht and Aster, 2000). It is invariably associated with chromosomal translocations, preferentially the t(8:14)(q24;q32) translocation, bringing the c-MYC proto-oncogene in proximity with the immunoglobulin heavy chain promoter (Dalla-Favera et al., 1982; Taub et al., 1982). Even though the chromosomal breakpoints are widely dispersed along the genes, the end result is a fusion gene where c-MYC is constitutively active. Because of the c-MYC overexpression, BL cells have the highest cell division rate observed in any human tumor. Although c-MYC rearrangements are observed in the majority of BL cases, this is not a BL-specific phenomenon as they are also observed in other types of lymphoma (reviewed in Hecht and Aster, 2000). However, translocated and activated MYC is the consistent feature of these
tumors and not the presence of EBV. The exact mechanism of the MYC translocations seen in BL and other lymphomas has for a long time been a puzzle. Recently, AID (activation-induced cytidine deaminase) that is highly expressed in the lymph node germinal centre and can be induced by EBV late proteins was shown to be essential for the MYC translocations. AID activation induces deamination of cytidine residues, resulting in U:G mismatches and double-stranded DNA breaks (Roughan and Thorley-Lawson, 2009). Aberrant AID expression in IL-6 transgenic mice causes Ig-MYC translocations that mimics those detected in EBV-positive BL (Dorsett et al., 2007).

D. Treatment of BL

Even though BL is a highly aggressive malignancy, it is highly treatable particularly in children and has a low level of relapse. The main treatment is chemotherapy, often consisting of various combinations of cyclophosphamide, vincristine, doxorubicin, methotrexate, cytarabine, ifosfamide, and etoposide (Aldoss et al., 2008; Lacasce et al., 2004; Mead et al., 2002). Cyclophosphamide and ifosfamide are both nitrogen mustard-derived alkylating agents used in treatment of lymphomas, leukemias, and some solid tumors (Shanafelt et al., 2007; Young et al., 2006). The drugs cause cell death by inducing DNA cross-links in cells with low levels of aldehyde dehydrogenase. Cyclophosphamide-induced activation of p53 along with a decrease in c-MYC and Odc expression cause cell-cycle arrest, at least in cells from the gastrointestinal tract (Hui et al., 2006). Doxorubicin and etoposide are both topoisomerase-II inhibitors (Hande, 1998; Rich et al., 2000). While doxorubicin is an anthracycline antibiotic, analogous to daunorubicin, with broad-spectrum antitumor effects, the podophyllotoxin etoposide was the first anticancer drug demonstrated to inhibit topoisomerase-II. Etoposide causes p53 phosphorylation, possibly mediated by the DNA damage sensor DNA-PK, with subsequent upregulation of proapoptotic Bax and promotion of apoptosis through cytochrome c release (Karpinich et al., 2002). Although poorly soluble in water and thus difficult to administer in effective doses, etoposide together with other topoisomerase-II-inhibiting agents are among the most effective chemotherapeutic drugs available for cancer therapy (Baldwin and Osheroff, 2005). The dihydrofolate reductase (DHFR) inhibitor methotrexate prevents DNA and RNA synthesis during the S phase of the cell cycle (Longo-Sorbello and Bertino, 2001). This antimetabolite is widely used in treatment of lymphomas, leukemias, some solid cancers, and also for autoimmune disorders. However, a high c-MYC expression was observed to cause methotrexate resistance in osteosarcoma and non-small cell lung cancer (NSCLC) cells (Scionti et al., 2008; Serra et al., 2008). The other antimetabolite, cytarabine (ara-C), causes DNA damage and inhibits DNA and
RNA synthesis in rapidly dividing cells (Grant, 1998). It is mainly used as an anticancer agent in treatment of lymphoma and leukemias, but also possesses antiviral activity (Gray et al., 1972). In leukemia cells, the effect of cytarabine was enhanced by inhibition of MEK signaling. The resulting DNA damage caused enhanced expression of p21 and downregulation of c-MYC and Bcl-XL, followed by growth arrest and apoptosis (Nishioka et al., 2009). Finally, vincristine is a vinca alkaloid antimicrotubule agent that prevents polymerization of tubulin (Jordan et al., 1991). Applications include treatment of lymphomas, childhood leukemia and it also functions as an immunosuppressant. Interestingly, the level of c-MYC does not appear to affect the cellular sensitivity to vincristine (Hirose and Kuroda, 1998; Ma et al., 1992).

Adult BL patients are often treated with monoclonal antibodies such as rituximab, combined with chemotherapy (Thomas et al., 2006). Rituximab targets the B cell surface antigen CD20 and is used for treatment of lymphomas, leukemias, and some autoimmune disorders. It is most efficient if the MYC gene is translocated to a non-Ig site, and if Bcl-2 protein expression is absent (Johnson et al., 2009). In cases where very high doses of chemotherapy are used, sometimes combined with radiotherapy, stem cell replacement may be required (reviewed in Aldoss et al., 2008). Stem cell transplantation may also be considered for patients with relapsed disease (Sweetenham et al., 1996). The use of selective serotonin-reuptake inhibitors (SSRI) has also been considered for their reported proapoptotic effect on B cell-derived tumors. However, this effect has not been proven to be specific (Schuster et al., 2007).

E. Lung Cancer

Lung cancer is one of the leading causes of cancer deaths in the industrialized world and has been correlated to cigarette smoking in over 80% of cases. Genetic factors and environmental exposures such as asbestos and radon contribute to the remaining 20% (Rom and Tchou-Wong, 2003). The disease has been divided into two histological subtypes in which the majority is NSCLC (80%) and the rest is SCLC. Both NSCLCs and SCLCs normally contain several numerical and structural chromosome alterations and epigenetic changes that result in aberrant expression of oncogenes and silencing of tumor suppressor genes. Lung cancer appears unique among epithelial tumors in that gene amplification and/or overexpression of each member of the MYC family, that is, c-MYC, MYCN, and MYCL can be detected in these tumors (Nau et al., 1985, 1986; Wong et al., 1986). MYC amplification occurs in 18–31% of SCLCs and in 8–20% of NSCLCs (Richardson and Johnson, 1993). Amplification of MYC genes has been shown to affect
survival adversely in SCLC patients (Brennan et al., 1991). The importance of MYC expression in the tumorigenesis of lung cancer was illustrated in transgenic mice expressing murine c-MYC under the control of the lung-specific surfactant protein C promoter. These mice invariably developed multifocal bronchiolo-alveolar adenocarcinomas from the alveolar epithelium (Ehrhardt et al., 2001).

Moreover, endogenous c-MYC is involved in nonmetastatic K-Ras-induced NSCLC as was shown by use of a dominant-negative c-MYC mutant (Soucek et al., 2008) and c-MYC in cooperation with c-Raf was recently shown to be a metastasis gene in NSCLC (Rapp et al., 2009). MYCN that is frequently amplified and expressed in SCLC (Nau et al., 1986) is crucial for normal lung organogenesis by maintaining a population of undifferentiated proliferating progenitor cells in the developing lung tissue. This is reflected by the findings that MYCN is highly expressed in embryonic lungs whereas adult lungs exhibit very low expression (Okubo et al., 2005). This in turn suggests that MYCN is involved in the maintenance of a population of continuously proliferating cells in the tumor, possessing similar properties as lung stem cells. Hence, targeting MYC in lung cancer may be an adjuvant therapy for the eradication of potential lung cancer stem cells.

F. Treatment of Lung Cancer

To date, there is no efficient treatment for lung cancer and much effort is being invested in improving patient survival and reducing the adverse effects of standard treatment. Systemic chemotherapy remains the most important treatment option for these patients (reviewed in Higgins and Ettinger, 2009). Treatment of NSCLC includes a combination of surgical resection and adjuvant chemotherapy consisting of platinum-based compounds such as cisplatin and carboplatin in combination with the nucleoside analogue gemcitabine or the antimitotic drugs paclitaxel, vinorelbine, or docetaxel; or the newer antifolate, pemetrexate (Blackhall et al., 2005; Higgins and Ettinger, 2009). In addition, the combination of chemotherapy and the anti-VEGF antibody bevacizumab is being investigated in relation to the effect of chemotherapy alone. Another approach that has received much attention is the use of small-molecule inhibitors of the epidermal growth factor receptor (EGFR) tyrosine kinase, overexpressed in more than 50% of all NSCLC cases (reviewed in Sharma et al., 2007). As EGFR overexpression is correlated with a bad prognosis, several drugs are in clinical trials and two molecules, Gefitinib and erlotinib, have been approved for NSCLC treatment. There is also the monoclonal EGFR antibody cetuximab that has shown promise in clinical trials (Higgins and Ettinger, 2009). As second and third line treatment options for NSCLC, proteasome inhibitors and
inhibitors of mTOR are being discussed along with sorafenib and sunitinib, kinase inhibitors with more general effects (Higgins and Ettinger, 2009). The traditional treatment of metastatic SCLC includes four different combinations: cyclophosphamide, doxorubicin, and vincristine; cisplatin and etoposide; ifosfamide and etoposide; and carboplatin and etoposide (reviewed in Wolf et al., 2004). Similarly to NSCLC, pemetrexate is also successfully applied in SCLC, as is the novel anthracycline drug amrubicin (reviewed in Higgins and Ettinger, 2009). The advancement in identifying markers for molecular targeted therapeutics will bring about a new era of personalized medicine where it is no longer appropriate to differentiate between NSCLC and SCLC.

In addition to K-Ras and EGFR, MYC appears to be a suitable marker that can be targeted by gene therapy in treatment of SCLC where MYC is overexpressed. Cells transduced with the herpes simplex virus thymidine kinase (HSV-TK) expressed from the E-box sequence (CACGTG) had an increased sensitivity to ganciclovir in cells overexpressing MYC (Nishino et al., 2001). Moreover, when these motifs were placed in a replication-deficient adenoviral vector (adMYCTK) and injected in MYC-overexpressing tumors in mice, followed by ganciclovir-administration, the tumor size was markedly reduced. This ganciclovir-induced shrinkage was not observed in adMYCTK-infected tumors not overexpressing MYC (Nishino et al., 2001). As this treatment rendered no apparent side effects, it may be useful for clinical purposes in patients with MYC-overexpressing SCLC refractory to standard treatment.

G. Medulloblastoma

Medulloblastoma, a primitive neuroectodermal tumor, is the most common malignant pediatric brain tumor. It arises in the cerebellum and can originate from cerebellar granule neural precursor (GNP) cells located in the external granular layer (EGL) of the cerebellum (Schuller et al., 2008; Yang et al., 2008). The EGL contains actively proliferating progenitor cells derived from the rhombic lip during embryogenesis. While GNP cell proliferation requires Hedgehog signaling (Ho and Scott, 2002), their expansion and survival is also promoted by IGF signaling. Medulloblastoma cells retain many features resembling precursor cells of the embryonic brain (Schuller et al., 2008) and more than half of these tumors contain abnormal activation of the Hedgehog or Wnt signaling pathways (Hambardzumyan et al., 2008b). Moreover, activation of the PI3K/Akt signaling pathway has been shown to be important for proliferation of human medulloblastoma cells and cancer stem cells residing in the perivascular niche following radiation of medulloblastoma (Hambardzumyan et al., 2008a; Hartmann et al., 2006; Rao et al., 2004).

Elevated MYCN expression is present in a significant proportion of human medulloblastoma (Eberhart et al., 2004; Pomeroy et al., 2002), and
is required for Sonic hedgehog-driven medulloblastoma tumorigenesis (Hatton *et al*., 2006). Activation of the *c*-MYC oncogene is frequently observed in medulloblastoma and has been shown to be one of the most reliable prognostic factors (Eberhart *et al*., 2004; Herms *et al*., 2000). Moreover, activation of both the Wnt/β-catenin pathway as well as of PI3K/Akt signaling have been shown to affect the expression of both MYCN and *c*-MYC in medulloblastoma cells (Baryawno *et al*., 2010; Browd *et al*., 2006; Momota *et al*., 2008). Hence, MYC appears to play a central role in mediating the effects of aberrant Hedgehog, Wnt, and PI3K/Akt signaling in medulloblastoma.

**H. Treatment of Medulloblastoma**

The standard treatment of medulloblastoma still consists of surgery followed by high-grade craniospinal radiotherapy (reviewed in Mueller and Chang, 2009). However, as this treatment causes severe morbidity to the relatively few (<60%) surviving children, there are several ongoing trials in search of milder, but more efficient treatments. Single agent chemotherapy as well as treatment with a combination of drugs have been tested with varying success. Despite favorable initial responses to drugs such as methotrexate, cyclophosphamide, platinum drugs, vincristine, ifosfamide, etoposide, and temozolomide in most cases, the long-term disease control rate did not increase, suggesting that chemotherapy alone would not provide a cure for the disease. One obvious problem in using chemotherapy in treating medulloblastoma is the limited ability for many of the drugs to cross the blood–brain barrier. Therefore, chemotherapy is combined with radiation to give the best response and to reduce morbidity and mortality.

There are several preclinical and clinical trials in search of targeted therapies for medulloblastoma (reviewed in Rossi *et al*., 2008). The two treatment approaches that have reached clinical trials target either the sonic hedgehog pathway or the EGF tyrosine kinases. The hedgehog targeting therapy is represented by the teratogen cyclopamine that binds to and inactivates the smoothened protein. The displayed *in vitro* outcomes of this treatment were cell-cycle arrest, initiation of neuronal cell differentiation, and consequently loss of the stem cell-like characteristics (Berman *et al*., 2002; Romer and Curran, 2005). It appears that this effect occurs specifically in medulloblastoma-derived cells and not in cells resected from other brain tumors. Also, GDC-0449, a compound that inhibits Hedgehog signaling by deactivation of Smoothened has been used in the treatment of medulloblastoma with very good responses initially. However, the patient developed resistance against GDC-0449 caused by a point mutation in *Smoothened* preventing the compound to lock into the Smoothened protein (Rudin *et al*., 2006).
Targeting of epidermal growth factor tyrosine kinases (Erbb1 and -2) prevents the invasiveness of medulloblastomas. Therefore, small-molecule tyrosine kinase inhibitors have been designed to target Erbb1 and Erbb2. As such, the Erbb2 targeting drug erlotinib selectively blocks upregulation of prometastatic genes such as S100A4 (Hernan et al., 2003), and is being tested for treatment of refractory solid brain tumors in combination with either radiation (clinicaltrials.gov: NCT00360854) or in combination with the alkylating agent temozolomide (Jakacki et al., 2008). The latter combination is also a potential new therapy for neuroblastoma.

Another recent approach using the telomestatin derivative S2T1-6OTD, targeting G-quadruplex forming DNA sequences, proved to potently inhibit c-MYC transcription and its target gene hTERT (Shalaby et al., 2010). The effective dose of the small molecule induced telomere shortening and cell-cycle arrest by downregulating Cdk2 protein expression in childhood brain cancer cell lines, including medulloblastoma. If this compound proves effective in animal models, it represents a promising new therapeutic approach. Effects of targeting c-MYC by RNA interference techniques have also been investigated (von Bueren et al., 2009). In a panel of human medulloblastoma cell lines, it was found that in addition to inhibiting cellular proliferation and clonogenic growth, c-MYC downregulation also reduced the apoptotic response to chemotherapy and radiation. This approach would therefore require a timely introduction in combination with other therapies.

I. Neuroblastoma

Neuroblastoma is a malignant embryonal tumor of the peripheral nervous system that accounts for more than 10% of pediatric cancer deaths despite intensive treatment modalities (Johnsen et al., 2009). Most cases are diagnosed during the first year of life at the peak of disease incidence (reviewed in Hogarty, 2003). The clinical outcome is heterogenous, ranging from spontaneously regressing tumors, to differentiating tumors and those that can be cured with chemotherapy, but also includes cases of aggressive metastatic tumors, often associated with a lethal outcome (Weinstein et al., 2003). Neuroblastoma originates from neural crest cells and is linked to dysfunctional pathways, which are operative during normal development (Scotting et al., 2005). The neural crest is a transient embryonal structure that arises from ectoderm during closure of the neural tube. A complex interplay between Hedgehog and Wnt signaling is important for proper neural crest formation (Fodde and Brabletz, 2007). Both Hedgehog and Wnt signaling have been shown to induce expression of MYCN. High MYCN expression stimulates proliferation and migration of neuroblasts, while reduced levels of this protein is associated with terminal differentiation.
Amplification of the MYCN gene, which occurs in 40–50% of high-risk neuroblastoma cases, remains the major key predictor of poor outcome and is associated with advanced-stage disease, rapid tumor progression, and a low survival rate (Maris et al., 2007). This suggests an important function of MYCN in neuroblastoma. In fact, transgenic mice with targeted expression of MYCN to neural crest cells using the tyrosine hydroxylase promoter (pTH-MYCN) develop neuroblastoma that is histologically and genetically very similar to aggressive undifferentiated human neuroblastoma (Weiss et al., 1997). Interestingly, in MYCN nonamplified neuroblastomas the level of MYCN transcripts and proteins do not correlate with outcome (Cohn and Tweddle, 2004; Cohn et al., 2000; Tang et al., 2006). Instead it was recently shown that neuroblastoma cells with low MYCN levels frequently overexpress c-MYC (Westermann et al., 2008). Moreover, constitutive activation of PI3K/Akt as well as activation of Wnt signaling has recently been shown in primary neuroblastomas (Johnsen et al., 2008; Liu et al., 2008; Opel et al., 2007; and reviewed in Gustafson and Weiss, 2010). Activation of both these signaling pathways is associated with increased MYCN expression in neuroblastoma (Johnsen et al., 2008; Liu et al., 2008). This suggests that a common MYC-dependent transcriptional profile may contribute to the pathogenesis and that therapies targeting MYC expression may have importance in clinical outcome. In fact, pathway-specific gene expression profiling using two large neuroblastoma datasets showed that patients with poor prognosis, as well as all MYCN-amplified cases, had elevated signaling through the MYC transcriptional network (MYC, MYCN, and MYCL target genes). This in turn suggests that overexpression of MYC target genes contributes to neuroblastoma aggressiveness (Fredlund et al., 2008).

### J. Treatment of Neuroblastoma

Depending on the disease stage, the treatment approaches for neuroblastoma consist of different combinations of surgery, radiation therapy, chemotherapy, and simply watchful waiting. Standard chemotherapy regimens used in treatment of neuroblastoma include different combinations of cisplatin, vincristine, carboplatin, etoposide, and cyclophosphamide (Pearson et al., 2008). A recent study demonstrated a significantly better 5-year event-free survival in patients receiving myeloablative therapy with autologous bone marrow transplantation (Matthay et al., 2009). Subsequent treatment with 13-cis-retinoic acid (RA), causing cell growth arrest and differentiation of tumor cells, further improves the overall survival in children suffering from neuroblastoma. Previous results showed that one mechanism of 13-cis-RA was to reduce MYCN mRNA expression (Reynolds and Lemons, 2001).
Treatments in clinical trials comprise a combination of the monoclonal antibody CH14.18, targeting the tumor antigen GD2, and cytokines IL-2 and GM-CSF together with 13-cis-RA. Systemic distribution of IL-2 cytokines is used to activate natural killer cells and a certain subpopulation of T cells into lysing the antibody-coated neuroblastoma cells (Verneris and Wagner, 2007). In the humanized antibody hu14.18-IL-2, a humanized version of CH14.18 has been coupled to recombinant IL-2. Other immune-based therapies in clinical trials include ex vivo activated and expanded T cells, tumor cell vaccines, tumor pulsed dendritic cells, and allogeneic HSC transplants; all requiring quite extensive further investigations before they can be approved for neuroblastoma treatment (reviewed in Verneris and Wagner, 2007). A synthetic vitamin A derivative, Fenretidine, reduces angiogenesis by inhibiting migration of endothelial cells and reduces the growth of neuroblastoma in vitro (Friedman and Castleberry, 2007). Another angiogenesis inhibitor undergoing clinical trials is TNP-470, a synthetic peptide that seems to work best in patients with a small tumor burden (Shusterman et al., 2001). As such, TNP-470 has been suggested for use in treatment of minimal residual disease after chemotherapy. The recent discovery of anaplastic lymphoma kinase (Alk) mutations and/or amplification in high-risk neuroblastoma and the findings that small-molecule inhibitors of Alk suppress neuroblastoma growth in vitro and in vivo have resulted in a clinical trial using PF-02341066, a c-Met inhibitor that also has significant activity against Alk (Mosse et al., 2009).

Among many other features causing drug resistance, MYCN may be responsible for neuroblastoma cell resistance to vincristine and cisplatin (Blaheta et al., 2007). Therefore, MYCN together with oncogenes such as MDM2 and ALK comprise potential new targets for molecular intervention in future neuroblastoma treatment (reviewed in Van Roy et al., 2009). In addition to targeting ALK, trials are also ongoing where inhibitors of PI3K (SF1126), the Trk neurotropin receptor (CEP-701), and the Aurora A kinase (MLN8237) are being evaluated (reviewed in Gustafson and Weiss, 2010). One MYCN targeting approach still awaiting clinical trials is the employment of peptide nucleic acids (PNAs), DNA analogs modified for a higher stability and longer duration of activity (reviewed in Morgenstern and Anderson, 2006). An antisense MYCN PNA conjugated to a somatostatin analog was demonstrated to be rapidly internalized and significantly inhibited cell growth of neuroblastoma cells (Sun et al., 2002). An even better outcome was observed by the use of antigene PNAs, designed to be complementary to the coding DNA strand (Tonelli et al., 2005). These molecules block gene expression at the transcriptional level and were shown to inhibit cellular proliferation in a panel of neuroblastoma cell lines at a similar rate as the observed reduction in MYCN expression. Additional preclinical investigations involve the use of small-molecule inhibitors, antisense oligonucleotides, and miRNA (described below).
K. Rhabdomyosarcoma (RMS)

RMS represents the most common pediatric soft tissue sarcoma. The sarcomas resemble developing skeletal muscle and are, based on histology, divided into the two main subtypes alveolar and embryonic RMS (Anderson et al., 1999). The tumors can be distributed to nearly any tissue in the body, except bone, but the head and neck area and the genitourinary tract are the most common locations in children (reviewed in Hayes-Jordan and Andrassy, 2009). Markers for RMS include transcription factors in skeletal muscle; differentiation and structural proteins normally seen in mature skeletal muscle. While embryonic RMS represents the majority of cases (~75%), the worst prognosis is observed in patients with alveolar RMS (reviewed in Morgenstern and Anderson, 2006). A complicating factor in diagnosing the disease is the lack of serum markers. Therefore, open biopsies are often required in order to confirm the RMS (Hayes-Jordan and Andrassy, 2009). One potential prognostic factor is MYCN that has been detected in increased copy numbers in both the embryonic and the alveolar subtype. In the alveolar subtype, overexpression or gain of genomic copies of MYCN has been significantly associated with adverse outcome (Williamson et al., 2005). In contrast, high genomic copy number of MYCN did not necessarily lead to high protein expression and MYCN amplification did not correlate with clinical outcome in the embryonic variant.

L. Treatment of RMS

There are basically three different international therapeutic protocols, depending on the risk of recurrence: Low risk (estimated 3-year failure-free survival (FFS) rate of 88%), intermediate risk (estimated 3-year FFS rate: 55–76%), and high risk (estimated FFS rate < 30%) (Hayes-Jordan and Andrassy, 2009). A multimodality approach is required, but while low-risk patients are treated with relatively low doses of radiation and chemotherapy those in the intermediate risk-group receive a combination of chemotherapeutic drugs combined with radiation when possible (reviewed in Hayes-Jordan and Andrassy, 2009). Surgical excision of the tumor is performed in conjunction with chemotherapy where commonly used drugs include: vincristine, actinomycin, cyclophosphamide/ifosfamide, and irinotecan (Hayes-Jordan and Andrassy, 2009). In high-risk cases, particularly that of alveolar subtype, there is a clear need for new treatment strategies (Morgenstern and Anderson, 2006). As nearly 25% of patients with alveolar RMS display tumors with MYCN deregulations, there are strong implications for targeting MYCN in this subtype.
There are several new potential treatment approaches for RMS, most of which are still at the preclinical stage (reviewed in Morgenstern and Anderson, 2006). One potential target for such therapies is IGF and its receptor. The approaches include both monoclonal antibody therapy and selective inhibitors of the receptor tyrosine kinase (Garcia-Echeverria et al., 2004; Maloney et al., 2003). Immunotherapy techniques comprise another potential future strategy aiming to specifically target the tumor cells and produce fewer, less severe side effects (Morgenstern and Anderson, 2006). As an example, there have been attempts at “priming” cytotoxic T lymphocytes (CTLs) into targeting the alveolar RMS-specific protein Pax3-Foxo1A (Mackall et al., 2000). Similar attempts at producing CTLs specifically targeting and killing MYCN-amplified neuroblastoma cell lines may also present a useful alternative in RMS treatment (Sarkar and Nuchtern, 2000). The potential efficacy of a vaccination approach is also being evaluated (reviewed in Morgenstern and Anderson, 2006). Other strategies under investigation for targeting deregulated MYCN in tumors include the potential use of PNAs investigated for neuroblastoma treatment, small-molecule inhibitors, and antisense oligonucleotides.

IX. NOVEL THERAPIES

The most frequently used anticancer drugs, including chemotherapeutics targeting topoisomerases, DNA-damaging agents, mitotic inhibitors, antimetabolites, and nucleotide analogues, suffer the disadvantage of causing resistance development (Herr and Debatin, 2001; Luqmani, 2005). This is most likely due to a deficient apoptotic program in tumor cells together with increased efflux and decreased influx of the drug, and increased DNA repair. In addition, the adverse effects such as induction of myelotoxicity, nausea, vomiting, diarrhea, and fatigue often caused by these agents (Nieboer et al., 2005) calls for novel treatments less prone to cause side effects and resistance development in the patients.

A. Rational Design and Synthetic Modeling: Successful Examples

Screenings aimed to find molecules targeting the kinase domain of tumor-associated proteins have resulted in the development of the phenylamino-pyrimidine-derivative imatinib mesylate (Gleevec™/STI-571). This compound targets the kinase domain of the fusion protein Bcr-Abl (Druker, 2002), and was later found to inhibit other kinases, such as the stem cell factor receptor c-Kit and PDGFR (Druker, 2002; Nadal and
Olavarria, 2004). Gleevec is currently used in treatment of chronic myeloid leukemia (CML) ( Nicolini et al., 2006) and gastrointestinal stromal tumors (GIST) ( von Mehren, 2006), and is also undergoing a number of clinical trials as adjuvant treatment of refractory or metastatic solid tumors (http://www.cancer.gov/clinicaltrials).

In a more direct approach, the three-dimensional structure of crystallized protein(s) is used for modeling site-specific compounds by computer-based predictions. These compounds are then synthesized for analysis of their biological activity (Kontopidis et al., 2003; McClue et al., 2002). The highly specific Cdk2-Cyclin E-targeting compound R-roscovitine (Seliciclib/CYC202), currently undergoing clinical trials (Benson et al., 2007), was identified using this approach (De Azevedo et al., 1997; Meijer et al., 1997) and has been found to significantly reduce tumor size in colorectal xenograft mouse models (McLaughlin et al., 2003; Raynaud et al., 2005). However, in spite of these exact measures to engineer the perfect anticancer drug, it remains difficult to find a compound for which the mechanisms of action can be exclusively specified.

Yet another strategy for identifying new treatments includes screening of low-molecular compound libraries in search for substances eliciting target-specific antiproliferative or proapoptotic effects. This method was successfully applied in identification of PRIMA-1 (p53-reactivation and induction of massive apoptosis) (Bykov et al., 2002), a molecule that has been found to enhance the apoptosis of agents such as cisplatin and doxorubicin (Bykov et al., 2005; Magrini et al., 2008) and for which the mechanism of action is currently being elucidated (Lambert et al., 2009). Similarly, molecular screens for compounds interfering with transactivation by c-MYC or MYCN or with MYC/Max heterodimerization have been successful, yielding candidate compounds awaiting further investigation (Berg et al., 2002; Hueber and Evan, 1998; Lu et al., 2003; Xu et al., 2006; Yin et al., 2003).

Below, we outline some examples of novel therapies in clinical use or in preclinical studies, as well as promising approaches to bring forth MYC pathway-specific anticancer treatments.

X. TARGETED THERAPY: WHAT IS IN THE FUTURE FOR MYC?

Targeting MYC or the MYC pathway has emerged as a very attractive approach to search for cancer intervention. This is because MYC is frequently deregulated in human tumors and is even believed to be aberrantly expressed in a major fraction of all cancers (Hermeking, 2003; Pelengaris and Khan, 2003; Prochownik, 2004). Several new strategies are being
investigated, some of which are more promising than others (Dang et al., 2009; Hermeking, 2003; Johnsen et al., 2009; Lu et al., 2003; Pelengaris and Khan, 2003; Prochownik, 2004; Vita and Henriksson, 2006). Here, we present some of the many approaches for targeting MYC at different levels (summarized with references in Table II). However, we did not bring up implications for therapeutic interventions of MYC-mediated energy metabolism since this issue was reviewed recently by experts in the field (Dang et al., 2009).

A. Substances Interfering with the MYC Pathway

The MYC pathway could be targeted either directly by tackling the MYC protein itself or indirectly by affecting upstream regulators or downstream effectors. MYC protein expression could be controlled by affecting the stability or degradation while its activity could be regulated by affecting the dimerization capacity or the DNA-binding ability (Fig. 3). Several approaches including different screening assays have been used in order to identify substances that control MYC expression or activity (Berg et al., 2002; Lu et al., 2003; Mo and Henriksson, 2006; Xu et al., 2006; Yin et al., 2003).

In search for substances affecting MYCN-mediated transactivation, Lu et al. utilized a luciferase screening assay in neuroblastoma cells where the MYC target gene ODC served as reporter (Lu et al., 2003). From a library of 2800 compounds, they identified eight compounds that significantly inhibited MYC-induced luciferase activity, five of which showed MYCN-specificity. These substances are being further evaluated for their potential use as lead substances (Lu et al., 2003). Our lab employed a cellular screening strategy in search for MYC pathway response agents (MYRAs) (Mo and Henriksson, 2006). Using cells with conditional c-MYC expression, we selected substances that affected viability in c-MYC-overexpressing cells. Two substances, MYRA-A and MYRA-B, were found to induce apoptosis and inhibit transformation in a MYC-dependent manner without affecting MYC/Max dimerization. Together with a third substance from the initial screen, they were also found to target MYCN overexpressing cells, suggesting their potential use in treatment of both c-MYC and MYCN overexpressing tumors (Mo et al., 2006). A different approach was taken in a screen for oncogenic pathways responsive to Cdk1 inhibition (Goga et al., 2007). Cells transformed with MYC were found to be sensitized to apoptosis in response to treatment with the Cdk1 inhibitor purvalanol in contrast to those transformed by other oncogenes. As this was independent of the p53–MDM2–ARF pathway, and appeared to be specific for MYC, Cdk1 inhibition has been suggested as a future therapeutic model for human malignancies overexpressing MYC. However, this requires identification of a Cdk1 inhibitor better suitable for
in vivo treatment, as purvalanol does not dissolve well in aqueous solutions (Goga et al., 2007). Another possibility would be to target survivin, a molecule affecting apoptosis and mitotic spindle functions and that is often overexpressed in human cancers (Mita et al., 2008). There have been numerous

<table>
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<th>MYC-specific therapy</th>
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<td>MYCN antisense and antigene peptide nucleic acids (PNAs)</td>
<td>Sun et al. (2002), Tonelli et al. (2005), Morgenstern and Anderson (2006; review)</td>
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<td>Disrupting MYC/Max dimerization by 10058-F4 and its analogues + 10058-F4-related molecules</td>
<td>Yin et al. (2003), Huang et al. (2006), Lin et al. (2007), Wang et al. (2007), Follis et al. (2008), Guo et al. (2009), Hammoudeh et al. (2009)</td>
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<td>Small-molecule inhibitors other than 10058-F4 (MYRAs, IIA6B17, and others)</td>
<td>Berg et al. (2002), Lu et al. (2003), Mo and Henriksson (2006), Mo et al. (2006), Xu et al. (2006), Lu et al. (2008)</td>
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<td>Interfering with MYC-induced energy metabolism</td>
<td>Dang et al. (2009); review</td>
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<td>Cdk1 or Cdk2 inhibition</td>
<td>Goga et al. (2007), Hydbring et al. (2009), Campaner et al. (2010)</td>
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<td>Survivin targeting (such as shepherdin)</td>
<td>Mita et al. (2008), Plescia et al. (2005)</td>
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<td>Histone deacetylase inhibitors (trichostatin-A and others)</td>
<td>McLaughlin et al. (2003), Albihn et al. (unpublished data)</td>
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<td>G-quadruplex DNA stabilizers (TMPyP4 porphyrin, S2T1-6OTD telomestatin derivative)</td>
<td>Grand et al. (2002), Shalaby et al. (2010)</td>
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<td>Targeting of miRNAs (let7, miR-17-92, miR 15a, miR-34a)</td>
<td>He et al. (2005), O’Donnell et al. (2005), Sampson et al. (2007), Chang et al. (2008), Cole et al. (2008), Leucci et al. (2008), Shi et al. (2008; review), Loven et al. (2010)</td>
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<td>MYCN-targeting cytotoxic T cells (CTLs), vaccination approaches</td>
<td>Sarkar and Nuchtern (2000), Morgenstern and Anderson (2006; review)</td>
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<td>Gene therapy using MYC-thymidine kinase expressing adenoviral vector (adMYCTK)</td>
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<td>Interfering with upstream regulation of MYC (USP28, CIP2A, PI3K/Akt)</td>
<td>Chesler et al. (2006), Popov et al. (2007), Johnsen et al. (2008), Juntila et al. (2007, 2008), Baryawno et al. (2009), Khanna et al. (2009)</td>
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<td>Manipulating MYC target proteins (Bcl-2 proteins, ODC, etc.)</td>
<td>Mason et al. (2008), Nilsson et al. (2005), Raul (2007; review), Shantz and Levin (2007), Hogarty et al. (2008), Evageliou and Hogarty (2009)</td>
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<td>Transient MYC inactivation (Tet-regulated, ER-regulated, Omomyc)</td>
<td>Felscher and Bishop (1999a,b), Pelengaris et al. (1999), Soucek et al. (2008)</td>
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approaches to target this inhibitor of apoptosis protein (IAP) that is partially controlled by Cdk1 and also promotes some of its functions through interaction with Hsp90 (Altieri, 2008). A small peptide, shepherdin, engineered by rational design to prevent Survivin’s interaction with Hsp90, has been found to induce extensive apoptosis in tumor cells and in tumors in mice where it was well tolerated and did not induce significant signs of toxicity (Plescia et al., 2005).

As previously mentioned, targeting Cdk2 is another possible future therapeutic approach for MYC-overexpressing tumors, as this kinase appears to regulate MYC protein stability by phosphorylation at the S62 site (Hydbring et al., 2009). Indeed, Cdk2 was recently shown to prevent MYC-induced cellular senescence (Campaner et al., 2010). In addition, it was found that pharmacological inhibition of Cdk2 induced MYC-dependent senescence independently of p53 and without enhancing MYC-driven replication stress.

Histone Deacetylase (HDAC) inhibitors became interesting as targets for cancer therapy when it was found that they could also control deacetylation of proteins other than histones (McLaughlin et al., 2003) and several HDAC inhibitory compounds are already in clinical trials. In addition, we have shown that Trichostatin-A efficiently kills cells with MYC overexpression suggesting that HDAC inhibitors may be efficient in MYC-overexpressing tumors (Albihn et al., unpublished data).

1. INTERFERING WITH THE UPSTREAM SIGNAL

As tumor cell proliferation appears to require USP28-mediated MYC stabilization in many cases, this deubiquitinating enzyme (DUB) is viewed as an attractive target for future therapeutic tumor intervention (Junttila and Westermarck, 2008; Popov et al., 2007). USP28 appears to be essential for MYC-induced tumorigenesis and is highly expressed in colon and breast carcinomas. Since it belongs to a class of enzymes that can be selectively targeted, small molecules could be designed to block the USP28 activity (Popov et al., 2007). It has been suggested that inhibition of CIP2A would provide a possible therapeutic approach in treatment of certain cancer forms (Junttila and Westermark, 2008; Junttila et al., 2007). Indeed, CIP2A has been found overexpressed in head and neck cancer and colon cancer, and it also appeared that its depletion would cause degradation of the MYC protein. A prognostic role for CIP2A was suggested in gastric cancer where subgroups of patients, immunopositive for CIP2A, were found to have a reduced survival rate (Khanna et al., 2009). The investigators identified a positive-feedback loop between CIP2A and MYC, suggesting that MYC would directly promote gene expression of CIP2A, at the same time as CIP2A stabilized the c-MYC protein. This finding highlighted the potential benefit of targeting CIP2A as a therapeutic strategy as depletion of CIP2A would prevent anchorage-
independent growth as well as proliferation of the tumor cells. Similarly, inhibition of PI3K/Akt signaling has been shown to increase the degradation of MYCN in neuroblastoma (Chesler et al., 2006; Johnsen et al., 2008) and of c-MYC in medulloblastoma (Baryawno et al., 2010).

The Mnt protein should also be considered for future therapeutic strategies as it has been proposed to be a regulator of MYC activity. The possibility to enforce the repressive effect elicited by its potential tumor suppressor activity ought to be further explored (reviewed in Wahlstrom and Henriksson, 2007).

2. DISRUPTING MYC/MAX DIMERIZATION

Several screening projects have been performed with the aim to identify substances interfering with MYC/Max dimerization. In one of these studies, 10,000 substances were screened using the yeast two-hybrid assay, and seven molecules were found to specifically disrupt c-MYC/Max dimerization (Yin et al., 2003). All seven compounds were found to inhibit MYC-mediated transactivation and four of them also prevented tumor formation when cells that had been incubated with the compound for 3 days in vitro were inoculated into nude mice. One substance, 10058-F4 has been further studied and found to affect cellular apoptosis, differentiation, and cell-cycle progression in addition to its effect on the MYC/Max complex (Huang et al., 2006; Lin et al., 2007). Thus, this molecule showed great promise for further development, but turned out to be highly unstable and was rapidly degraded in vivo (Guo et al., 2009). This problem, together with the fact that the original 10058-F4 molecule had quite low potency, called for a search for more efficient analogues. By modification of the two ring structures in the quite simple 10058-F4 backbone, Wang et al. created second and third generation analogues of the molecule which proved to be more stable and efficient than the original compound (Wang et al., 2007). Recently, the binding site for 10058-F4 was located to the HLHZip region of the MYC protein, and it was found that three of the seven molecules indentified in the original screen could bind simultaneously to distinct sites of c-MYC without affecting the activity of the others (Follis et al., 2008; Hammoudeh et al., 2009).

Another technique, fluorescence resonance energy transfer (FRET) where the two proteins to be investigated are coupled to different color fluorescent proteins, is based on the color change, measured as a change in excitation wavelength, as the proteins connect. This approach was used by Berg et al. who identified two compounds from a library of 7000 that specifically interfered with MYC-induced oncogenic transformation of chicken embryo fibroblasts in culture (Berg et al., 2002). One of those molecules (IIA6B17) was recently found to specifically disrupt the transcriptional activity of c-MYC but not that of MYCN (Lu et al., 2008). This finding suggested
that a cell-based MYC luciferase reporter gene assay could be used as a tool to distinguish whether the candidate molecules are specific for c-MYC MYCN, or nonselective to help select their appropriate future use. Xu et al. designed and synthesized a credit-card library of 285 substances and identified several compounds that disrupted c-MYC/Max dimerization (Xu et al., 2006). The designation credit-card comes from the planar structure of the molecules, two of which were also found to prevent MYC-induced oncogenic transformation in cell culture.

3. ATTACKING THE MYC TARGETS

MYC-induced apoptosis is mainly mediated through the mitochondria, stimulating the release of cytochrome c. Proapoptotic Bcl-2 family proteins are important for permeabilization of the mitochondria and antiapoptotic members prevent this event, thus disrupting MYC-induced apoptosis. Therefore, blocking Bcl-2 activity is a possible approach to enhance MYC-driven apoptosis. ABT-737, a small molecule that similarly to BH3-only proteins binds to and inactivates some, but not all, antiapoptotic Bcl-2 proteins, was found to enhance apoptosis in lymphomas induced by MYC in combination with Bcl-2 (Mason et al., 2008). This was observed in vivo using ABT-737 as a single agent, and the effect was further enhanced in combination with cyclophosphamide. However, there was no evident effect in lymphomas driven by MYC alone, suggesting that antagonizing Bcl-2 may be an efficient supplement to conventional therapy in treating MYC-driven lymphomas overexpressing Bcl-2 (Mason et al., 2008).

Other proteins mediating the MYC effect, such as Odc, Cad, and hTERT, may also be targeted as a treatment approach to reduce the effect of MYC activation. Of these, the association between MYC and gene expression appear strongest for Odc where overexpression has been observed in human cancers such as MYC-induced lymphoma and MYCN-driven neuroblastoma (Hogarty et al., 2008; Nilsson et al., 2005). Potential therapeutic strategies for targeting Odc include the Odc inhibitor α-difluoromethylornithine (DFMO) in combination with conventional chemotherapy (review in Raul, 2007). Preclinical data has also proven this agent useful in treatment of lymphoma, neuroblastoma, and other malignancies (reviewed in Evageliou and Hogarty, 2009; Shantz and Levin, 2007).

4. HAMPERING WITH MYC EXPRESSION LEVEL

Antisense oligodeoxynucleotides (ASOs) targeting MYC have been successfully tested in several in vitro and in vivo models (Kutryk et al., 2002 and reviewed in Morgenstern and Anderson, 2006; Pelengaris and Khan, 2003; Prochownik, 2004; Tamm, 2005). For instance, experiments have been
aiming at enhancing the efficacy of cisplatin in vitro (reviewed in Prochownik, 2004), and reducing the MYCN protein level, resulting in reduced cell division and increased differentiation (reviewed in Morgenstern and Anderson, 2006). In vivo mouse data have been positive, showing that MYCN ASOs significantly reduced tumor incidence as well as tumor mass (Burkhart et al., 2003). Similar results were shown for c-MYC ASOs where data from phase-I clinical trials indicate that their distribution is tolerated in healthy human subjects (Kutryk et al., 2002 and reviewed in Prochownik, 2004). In addition, a third generation antisense molecule, the c-MYC targeting phosphorodiamidate morpholino oligomer (PMO) AVI-4126, was successfully applied in a phase-I clinical trial for prostate cancer, suggesting this as a safe and promising new therapeutic approach (Iversen et al., 2003). However, the therapeutic efficacy of these molecules remains to be explored.

Cationic porphyrins such as TMPyP4 that stabilize DNA G-quadruplexes have also been shown to downregulate c-MYC expression (Grand et al., 2002). Because of their additional inhibitory effect on the hTERT activity, these molecules are being studied as potential anticancer agents. A similar effect is observed in response to the telomestatin derivative S2T1-6OTD (Shalaby et al., 2010).

5. MAKING USE OF THE NONCODING SEQUENCE

MicroRNAs (miRNAs), small noncoding RNA molecules that were initially identified in Caenorhabditis elegans (Lee et al., 1993; Wightman et al., 1993), measure 18–24 nucleotides and account for ~1% of known genes. They bind to and negatively regulate protein coding mRNAs and are believed to be present in all multicellular eukaryotes (Ambros, 2004; Bartel, 2004; John et al., 2004; Kent and Mendell, 2006; Shi et al., 2008). Several pieces of evidence suggest that many miRNAs function as tumor suppressors or oncogenes, regulating the expression of proteins important in tumorigenesis (reviewed in Kent and Mendell, 2006; Shi et al., 2008). This “cancerous” feature of miRNAs makes them attractive as potential therapeutic targets, and the prospect of using them as biomarkers is also being explored. MYC has mainly been associated to two of the cancerous miRNA clusters, namely the let7 family of tumor suppressors and the oncogenic miR-17-92 cluster (Chang et al., 2008; He et al., 2005; Loven et al., 2010; O’Donnell et al., 2005; Sampson et al., 2007; Shah et al., 2007). The let7 family members are poorly expressed in several cancer forms and experimental evidence has shown that their ectopic expression reduced cell proliferation, inhibited tumorigenesis, and in one case even reduced metastasis (reviewed in Shi et al., 2008). In addition to silencing MYC (Sampson et al., 2007), let7 family members have been found to silence Ras and genes involved in cell-cycle and cell division control (Johnson et al., 2005, 2007). This, together
with the finding that let-7 overexpression can reduce resistance to chemotherapy in lung cancer (Weidhaas et al., 2007), suggests a future important role of this miRNA cluster in clinical use. The miR-17-92 polycistron contains seven human miRNAs (Shi et al., 2008) and is strongly associated with lymphomas and several solid tumors including neuroblastoma (Dews et al., 2006; Hayashita et al., 2005; He et al., 2005; Loven et al., 2010; Ota et al., 2004; Volinia et al., 2006). Even though it is mostly viewed as an oncogenic cluster, the effects of individual miR-17-92 members are strictly cell type and context dependent. For instance, one study in a panel of breast cancer cell lines demonstrated a tumor suppressor function for miR-17-5p (Hossain et al., 2006). The first evidence of \textit{in vivo} oncogenic activity of the miR-17 cluster was demonstrated in E\mu-MYC-transgenic mice, showing cooperation between the miR-17 cluster and c-MYC (He et al., 2005). This result was further strengthened by the finding that transcription of the miR-17 cluster is directly activated by c-MYC (O’Donnell et al., 2005). In the same study the MYC target E2F1, promoting cell-cycle progression, was shown to be negatively regulated by miR-17-5p/20a of the miR-17 cluster. MYC thus has two levels of control of the proliferative signal through E2F1, directly at the transcriptional level and indirectly at the translational level by activation of the miR-17 polycistron (O’Donnell et al., 2005). The miR-17-92 cluster was also found to be important in induction of B cell lymphomas, as recent experiments in mice demonstrated that miR-19 was the key oncogenic component that promoted lymphomagenesis in cooperation with c-MYC (Mu et al., 2009; Olive et al., 2009). This effect was partially due to repression of the tumor suppressor \textit{pten} (Olive et al., 2009), strengthening the previous notion of its association with the miR-17 cluster (Lewis et al., 2003).

Another important finding is that c-MYC also represses the expression of several miRNAs (Chang et al., 2008; Loven et al., 2010). Among the down-regulated miRNAs were miR-15a, miR-34a, and let-7; all located in genomic regions often deleted in cancer. miR-15a targets the anti-apoptotic gene Bcl-2 (Cimmino et al., 2005), let7 family members target Ras (Johnson et al., 2005), and miR-34a has been shown to be regulated by p53 (Bommer et al., 2007; Chang et al., 2007). These alleged tumor suppressors were established to be directly regulated by MYC and shown to inhibit experimentally induced B cell lymphomas in mice (Chang et al., 2008). There is also experimental data suggesting that the miR-34a cluster is responsible for c-MYC deregulation in cases of BL lacking the classical c-MYC translocation (Leucci et al., 2008). In addition, it appears that miR-34a has a tumor suppressor function in neuroblastoma where it was found to regulate MYCN as well as Bcl-2 (Cole et al., 2008). Taken together, these data implicate that control of miRNA expression is of great importance in MYC-mediated tumorigenesis. The fact that systemic delivery of small RNA molecules has been proven possible in animals (Soutschek et al.,
suggests the possibility for future therapeutic strategies based on delivering MYC-repressed miRNAs to combat cancer. Furthermore, a miRNA-based therapeutic approach has the advantage over single gene therapy that it targets multiple downstream effectors and may therefore be more effective (Petrocca and Lieberman, 2009).

B. Transient Inactivation of MYC

The prospect of targeting c-MYC is complicated by its nearly ubiquitous expression in proliferating cells since such a central protein might be crucial for tissue regeneration. However, Soucek and colleagues recently showed that transgenic mice tolerated the effects of extended MYC inhibition while almost complete regression of their K-ras-induced lung tumors was observed (Soucek et al., 2008). MYC was silenced by conditional expression of a dominant interfering MYC bHLHZip dimerization domain mutant called Omomyc. Despite strong effects on proliferating tissues in the intestinal crypts and the skin, these were rapidly reverted upon restoration of normal MYC function with no apparent damage to the animals. This study, together with other experiments where c-MYC has been transiently inactivated in more localized compartments (Felsher and Bishop, 1999a; Pelengaris et al., 1999), provides evidence of the benefit of pharmacological inhibition of c-MYC. If successfully confirmed in human subjects, this may be the preferred approach in treating MYC-driven tumors in the future.

XI. CONCLUDING REMARKS

We have highlighted some of the most important MYC functions and presented an overview of current and future therapies for a few cancers with MYC gene activation. The need for new, more specific cancer therapies is met by an intense research activity using different approaches and strategies. In addition, aspects such as timing, cellular location, and dosing also have to be taken into account when designing novel anticancer treatments targeting MYC or the MYC pathway. Most likely a combination of different approaches, both novel and/or conventional, rather than one single agent, the magic bullet, will provide future cancer cures.

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