

***c-myc*: a double-headed Janus that regulates cell survival and death**

Review Article

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Summary

A paradox for cancer biology is represented by the fact that some oncogenes, including *c-myc*, provide an advantage to cancer cells by stimulating uncontrolled proliferation while, at the same time, they exert a pro-apoptotic activity. The prominent roles of *c-myc* and the relevance of phosphorylation and subcellular compartmentalization of *c-Myc* protein are described in this review, which focuses also the possible strategies to modulate (i.e. up- and down-regulate) the *c-myc* level. The gene expression targeted approach of *c-myc* modulation as anticancer therapeutic treatment is discussed.

I. Introduction

A. *c-myc*: a proto-oncogene with many functions

It is generally assumed that the efficacy of anticancer drugs may be related to cell proliferation control and/or to the activation of the apoptotic pathway(s). Among the mediators of such processes, the *c-myc* proto-oncogene controls the balance between proliferation and death, thus playing a crucial role in different cell pathways leading to opposite effects (Prendergast, 1999; Amati et al, 2001; Eisenman, 2001; Nasi et al, 2001; Pelengaris et al, 2002; Pelengaris and Khan, 2003). In this respect, *c-myc* could be represented as Janus, the old Roman deity with two faces who presides over everything by regulating cell proliferation and cell death (**Figure 1**).

A simplified view of the activities of *c-myc* is shown in **Figure 2**. In normal cells, *c-myc* expression is tightly controlled by mitogenic stimuli and appears to be necessary, and in some instances sufficient, to induce cells to enter the S phase of cell cycle and to proliferate, and to respond to differentiative stimuli (Hoffman and Liebermann, 1994). Translocation and amplification of the *c-myc* gene as well as increased half-life and overexpression of the oncoprotein, which have been observed in many tumors, promote tumorigenesis (Spencer and Groudine, 1991; Marcu et al, 1992).

Deregulation of *c-myc* occurring in a broad range of human cancers is often associated with poor prognosis (Pelengaris et al, 2002). The molecular mechanisms for the frequently observed deregulation of *c-myc* in human cancers could depend on the fact that *c-myc* overexpression may antagonize the pro-apoptotic function of p53 (Ceballos et al, 2000). *c-myc* controls or affects other processes relevant to tumorigenesis, e.g. it can promote transformation by its ability to induce the expression of telomerase, thus bypassing telomere erosion and facilitating immortalization (Drissi et al, 2001).

Different factors may regulate in distinct ways *c-myc*-promoted cell transformation (O'Hagan et al, 2000). Among them, Bim acts as a suppressor of Myc-induced lymphomagenesis (Egle et al, 2004); non-peptide antagonists of Myc/Max dimerization inhibit *c-myc*-induced transformation (Berg et al, 2002); the ATM-related domain of TRRAP protein, which is involved in transcriptional regulation and chromatin structure, modulates *c-myc*-dependent oncogenesis (Park et al, 2001).

B. *c-Myc*-interacting proteins

c-Myc protein is a member of the helix-loop-helix leucine zipper family of transcription factors that bind to a DNA motif called "E-box", which consists of the consensus sequence CACGTG. Efficient binding of *c-Myc*

to an E-box requires the heterodimerization with its partner Max, another member of this family. Myc function is antagonized by the Mad protein, which can also dimerize with Max and bind to E-boxes (Amati et al, 2001; Baudino and Cleveland, 2001; Zhou and Hurlin, 2001). Since the main activities of Myc strictly depend on its dimerization with Max, the inhibition of such interaction may affect different processes. Indeed, small molecules acting as inhibitors of Myc/Max dimerization

were effective in counteracting the oncogenic activity of Myc (Berg et al, 2002).

c-myc initiates a transcriptional program that controls hundred of genes belonging to different functional categories of *myc* targets. Some of them can be considered as direct targets, others are indirectly regulated. The investigation of the nature of the interaction among *c-Myc* network members revealed that it could be modulated through the formation of distinct sub-nuclear structures localized in specific compartments (Yin et al, 2001).

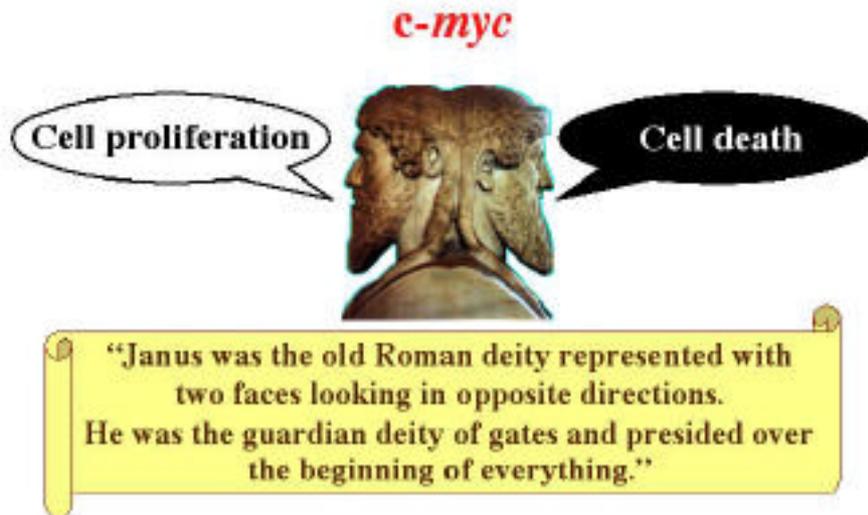


Figure 1. Representation of the oncogene *c-myc* as the double-headed Janus deity. Looking in the direction of both cell proliferation and death, *c-myc* controls the basic life processes.

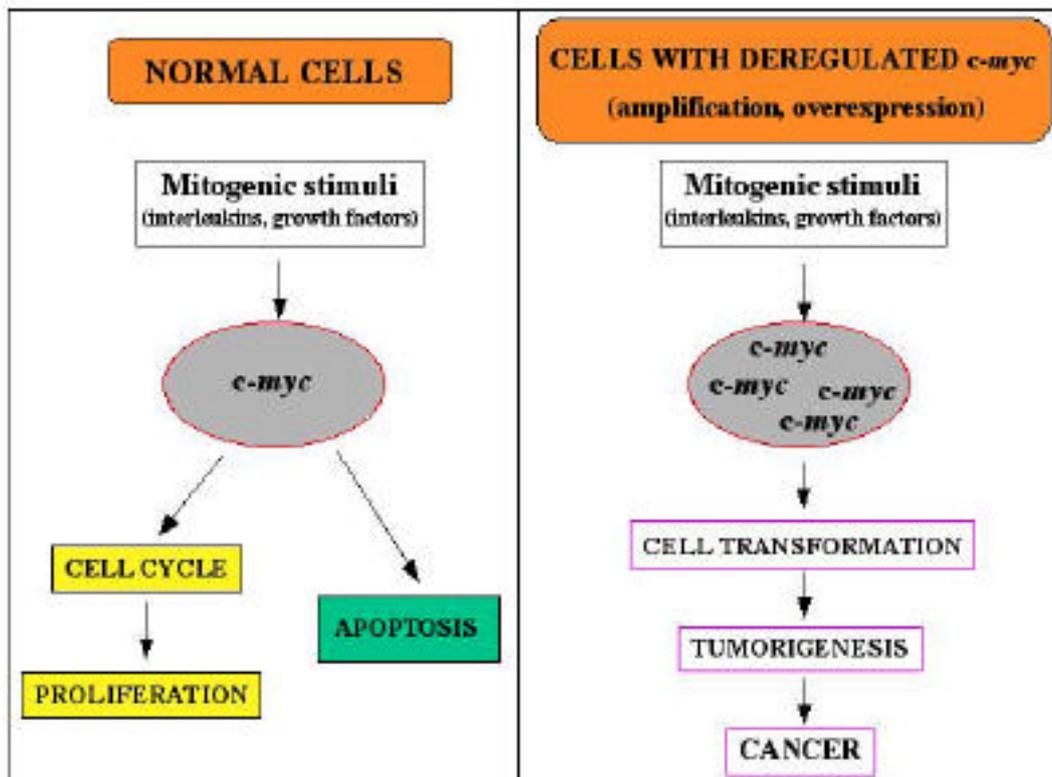


Figure 2. Regulation of different processes by *c-myc* in normal cells. Effect of *c-myc* deregulation in promoting cancer.

To date, the search for *c-myc* targets did not provide conclusive data. A still growing list of proteins regulated by *c-Myc* is reported and discussed in many reviews (Dang, 1999; Sakamuro and Prendergast, 1999; O'Hagan et al, 2000; Eisenman 2001; Levens, 2002, 2003; Fernandez et al, 2003; Nilsson and Cleveland, 2003, 2004; Patel et al, 2004). A variety of molecular, biological and genetic approaches were devised to identify the mRNAs induced or repressed by *c-myc*. Recent advances in proteomics and microarray technology allowed genome-wide studies of mRNA transcripts responsive to *c-Myc* (Schuhmacher et al, 2001; Shiio et al, 2002; Watson et al, 2002; Fernandez et al, 2003; Orian et al, 2003).

C. Regulation of apoptosis by *c-myc*

The observation that *c-myc* null fibroblasts are resistant to apoptosis highlighted the essential pro-apoptotic role of this oncogene (Chang et al, 2000). It is generally assumed that *c-myc* promotes apoptosis by sensitizing cells to a variety of insults rather than by acting as a direct death effector. Yu et al (2002) carried out a genome-wide survey for *myc*-mediated gene expression under apoptotic conditions. Isogenic Rat-1 cell lines that either overexpress or lack *c-myc*, were treated with etoposide, which induced apoptosis at an extent that depend upon the level of *c-myc*. The analysis provided the identification of a cluster of genes that respond to etoposide and are highly dependent on the cellular *myc* status. Moreover, the results revealed also that the existence of *c-myc*-independent genes involved in the apoptotic pathway.

Although a detailed understanding of the signalling pathways by which *c-myc* elicits apoptosis is still lacking, different factors have been shown to modulate *c-myc*-induced apoptosis. As first shown by Fanidi et al (1992) and Bissonnette et al (1992), the ability of *c-myc* to promote apoptosis can be suppressed by the overexpression of *bcl-2*; the same effect was obtained by the suppression of the pro-apoptotic factor Bax (Mitchell et al, 2000). Ionizing radiation-induced apoptosis can be increased by the activity of *c-Myc* in suppressing Bcl_{XL}, thus suggesting a strategy in desensitizing tumor cells to DNA damage-induced apoptosis (Maclean et al, 2003). The transcriptional repressor Mad1, which regulates negatively cell proliferation, has an inhibitory effect on *c-myc*-mediated apoptosis and proliferation (Gehring et al, 2000). Using RNA stable interference (siRNA), Nilsson and Cleveland (2004) showed that Mnt, a *myc* antagonist (Hurlin et al, 2004), triggers apoptosis via the *myc* target ODC. A similar indirect effect was described for the complex formed by the *c-myc*-negative regulator MBP-1 (*c-myc* promoter-binding protein 1), and MIP-2A (MBP-1-interacting protein), which in turn regulates negatively the MBP-1 activity and the induction of apoptosis (Ghosh et al, 2001).

A synergy between *c-myc* and different death receptors, leading to the release of cytochrome *c* from mitochondria, was shown (Kleffstrom et al, 2002). Remarkably, it has been reported that the gene for cytochrome *c*, which is required for apoptosis, is a direct target of *c-myc* and that *c-Myc* binds to it (Morrish et al,

2003). The analysis of the apoptosis induced in melanoma cells after *c-myc* down-regulation revealed that this process occurs through the specific depletion of the levels of glutathione (Biroccio et al, 2002).

In contrast to the pro-apoptotic function usually ascribed to *c-myc*, it has been shown that *c-myc* could contribute to block apoptosis under some conditions. In lymphoid CEM cells, treatment with oxysterols reduces *c-Myc* protein expression level before promoting apoptosis (Ayala-Torres et al, 1999), thus suggesting that the negative regulation of *c-Myc* does not inhibit the activation of apoptosis by steroid compounds.

D. *c-Myc* protein

c-Myc is a highly unstable phosphoprotein with a half-life of about 15-30 minutes. The phosphorylation sites Thr58 and Ser62 exert opposite effects on the control of *c-Myc* degradation through the ubiquitin-proteasome pathway (Flinn et al, 1998; Sears et al, 2000; Amati, 2004; Herbst et al, 2004; Welcker et al, 2004; Yeh et al, 2004). Recent data indicate that the stability of *c-Myc* is regulated by different sequence elements, i.e. the N-terminal "degron" that signals *Myc* ubiquitination and degradation, and the C-terminal "stabilon" that promotes its sequestration and stabilization into a subnuclear compartment (Herbst et al, 2004).

The N-terminal domain of *c-Myc*, which is essential for transcriptional and transforming activity, binds to α -tubulin (Alexandrova et al, 1995) and is released from it during mitosis to facilitate microtubule disassembly. The release of *c-Myc* from α -tubulin is regulated by *c-Myc* phosphorylation state (Noguchi et al, 1999; Gregory and Hann, 2000; Niklinski et al, 2000). *c-Myc* protein shows a predominant localization in the cytoplasm of interphase cells, while in proliferating cells its nuclear distribution is similar to that of some ribonucleoprotein (RNP)-containing structures (Spector et al, 1987), or is confined to large amorphous nuclear globules (Henriksson et al, 1988; Koskinen et al, 1991). The existence of a dynamic modification of *c-Myc* is suggested by the competition of phosphorylation and glycosylation for the same site, i.e. Thr58 (Kamemura et al, 2002).

The search for the precise intracellular localization of *c-Myc* in tumor cells, where its degradation is deregulated with a resulting abnormal stability of the protein in the nucleus (Flinn et al, 1998; Salghetti et al, 1999; Gregory and Hann, 2000; Niklinski et al, 2000; Herbst et al, 2004), revealed that phosphorylated *c-Myc* accumulates in the nucleus of tumor cells. Phosphorylated *c-Myc* is distributed in the form of spots of different sizes throughout the nucleus and in the nucleolus (Soldani et al, 2002), where *c-myc* transcripts were described (Bond and Wold, 1993). As clearly demonstrated in HeLa cells (Soldani et al, 2002), phosphorylated *c-Myc* does accumulate in large amorphous globules (Henriksson et al, 1998) and its distribution pattern is not reminiscent of the distribution of non-nucleolar RNP-containing structures, as reported by Spector et al (1987). Remarkably, in tumor cells treated with the antimitotic drug paclitaxel, the immunolabeling for phosphorylated *c-Myc* changed, and became more diffused throughout the nucleoplasm

(Bottone et al, 2003; Supino et al, unpublished observations). A typical example of the nuclear distribution of phosphorylated *c-Myc* in tumor cells is shown in **Figure 3**.

II. Strategies to modulate the *c-myc* level

A. Overexpression

The most common alteration affecting *c-myc* in human tumors is gene amplification (Nesbit et al, 1999), which can range from a single gene duplication to hundreds of copies. Many experiments based on the enforced expression of an exogenously introduced *c-myc* gene provided the evidence that *c-myc* amplification could sensitize tumor cells to apoptosis. The pro-apoptotic role for *c-myc* has been first shown in serum-starved primary or immortalized fibroblasts (Evan et al, 1992; Fanidi et al, 1992) and in IL-3-dependent myeloid cells upon withdrawal of the cytokine (Askew et al, 1991) and this role was further confirmed (Alarcon et al, 1996; Dong et al, 1977; Rupnow et al, 1998). Promising results have been obtained by Peltenburg et al (2004), who demonstrated that the stable transfection of IGR39D melanoma cells with *c-myc* causes a sensitization of tumor cells toward apoptosis.

Although it is well established that apoptosis can be induced by the enforced expression of exogenously introduced *c-myc* genes in several experimental systems, it is interesting to investigate whether constitutive overexpression of the resident *c-myc* gene in tumor cells is sufficient to induce apoptosis. A positive correlation between endogenous high level of *c-myc* and apoptosis propensity was found in lymphoblastic leukemic CEM cells, which harbor constitutive activation of *c-myc* and undergo serum starvation-induced apoptosis (Tiberio et al, 2001).

We addressed this question by examining the effect of different apoptogenic stimuli on tumorigenic and non-tumorigenic clones isolated from the SW613-S human

colon carcinoma cell line. 12A1 cells (tumorigenic clone) harbor an endogenous high level of amplification of the *c-myc* gene, whereas B3 cells (non-tumorigenic clone) have a small number of copies of this gene (Lavialle et al, 1988). We found that only cells with endogenous *c-myc* overexpression activate the apoptotic machinery in response to serum deprivation (Donzelli et al, 1999) and after the treatment with etoposide, doxorubicin and vitamin D₃, which induce Fas-mediated apoptosis (Gorrini et al, 2003). The low levels of *c-myc* expression present in SW613-B3 cells were unable to activate Fas-mediated apoptosis, thus suggesting that only a high *c-myc* expression can bypass the lack of Fas receptor. Apoptosis driven by DNA damage and long term-culture was independent of *c-myc* expression (Gorrini et al, 2003). The same experimental system was used to define the effect of *c-myc* amplification on the response to the antimetabolic drug paclitaxel. A high *c-myc* amplification level potentiates paclitaxel cytotoxicity, confers a multinucleated phenotype and promotes apoptosis to a high extent, thus suggesting that *c-myc* expression level is relevant in modulating the cellular responses to paclitaxel (Bottone et al, 2003).

In conclusion, the overexpression of *c-myc* could be a strategy for therapeutic applications, possibly by modulating *myc* levels, thus sensitising tumor cells to therapy. As an example of the clinical potential of the analysis of the *c-myc* expression level in tumors, recent data obtained on patients with ovarian cancer suggest that a high *c-myc* expression level could improve the chemotherapy response (Iba et al, 2004).

B. Inhibition

1. The gene expression targeted therapy

The identification of genes that are important for the development and maintenance of malignant phenotype opened new perspectives for eventually inducing a reversion to normal phenotype. In this view, disease-associated proteins can be targets of a selective therapy that would lead to less toxic side effects than the conventional, often cytotoxic, therapeutic treatment. In fact, the main limitation to conventional cancer chemotherapy derives from the lack of specificity of the drugs, and from pharmacokinetic and manufacturing problems, which can lead to systemic, and organ toxicity. This impairs the use of high-dose intensity therapy, giving rise to a high rate of tumor relapse. The identification of fundamental genetic differences between malignant and normal cells resulting, for example, from activated oncogenes and inactivated tumor suppressor genes, has made it possible to consider such genes as specific targets for antitumor therapy. In this respect, many genes have been selected for antisense therapy, including HER-2/neu, PKA, TGF- β , EGFR, TGF- β , IGFIR, P12, MDM2, BRCA, Bcl-2, ER, VEGF, MDR, ferritin, transferrin receptor, IRE, *c-fos*, HSP27, *c-myc*, *c-raf* and metallothioneins. Similar effects can be obtained with triple helix-forming oligonucleotides (TFOs) that are synthesized as to bind with a high affinity and specificity to double stranded DNA.

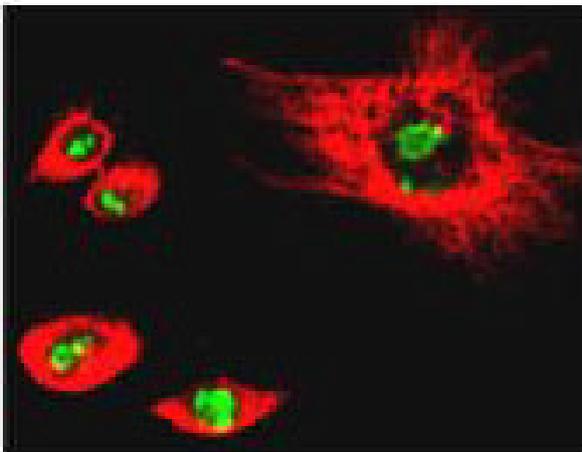


Figure 3. Nuclear localization of phosphorylated *c-Myc* in HeLa cells. Immunofluorescence experiments were carried out according to Bottone et al (2003). Red fluorescence: α -tubulin; green fluorescence: phosphorylated *c-Myc*.

2. Rational for the use of a therapy targeted against *c-myc*

Several genes known to be of importance in the regulation of apoptosis, cell growth, metastatization and angiogenesis provide a tantalizing prospect for the development of anticancer agents. Impaired apoptosis is a crucial step in tumorigenesis but is also a significant impediment to cytotoxic therapy (Hu and Kavanagh, 2003). Thus, agents targeted to interfere with appropriate molecules which regulate the apoptotic response to cell damage (spontaneous or induced by antitumor drugs) appear as a more rational therapeutic approach. As above reported, *c-myc* and *bcl-2* are important regulators of tumor progression and of apoptotic response to chemotherapy. Conflicting results have been reported on the role of *c-myc* expression in drug resistance (Leonetti et al, 1999; Knapp et al, 2003; Grassilli et al, 2004). Implication of *c-myc* in sensitizing cells to apoptosis in p53-mutant small cell lung carcinoma (Supino et al, 2001) and in prostate carcinoma cells (Cassinelli et al, 2004) has been reported. Thus, in tumors where overexpression of *c-myc* is related to drug resistance, a combined treatment with antitumor drugs and antisense oligonucleotides (AS-ODN) against *c-myc* could improve the therapeutic effects.

Additional approaches to modify *c-myc* expression consist of peptides, PNA (peptide nucleic acids) and siRNA (Cutrona et al, 2000; Hosono et al, 2004). Remarkably, it has been shown that c-Myc expression can be lowered by affecting the stabilization of a G-quadruplex structure present in the *c-myc* promoter (Grand et al, 2004).

3. Mechanism of action of antisense oligonucleotides and triple helix forming oligonucleotides

AS-ODN are able to inhibit specifically the synthesis of a particular protein by binding to protein-encoding RNA, thereby preventing RNA function and thus inhibiting the action of the gene. Antisense therapy should correct the mutations and abnormal expression of genes of tumor cells by decreasing their expression, inducing RNA degradation, and causing a premature termination of RNA transcription (Head et al, 2002). Oligonucleotides (ODNs) are short pieces of DNA; their size ranges generally from 18 to 21 nucleotides. They hybridize to a specific target mRNA and their action can be mediated by the cleavage of the target DNA or by blocking the translation of RNA. In the first case, once the AS-ODN is bound to the specific RNA target, cellular RNase H cleaves the RNA/ODN complex, cleaving the RNA strand and releasing the ODN which can bind another specific RNA strand. Alternatively, ODNs ribozymes can be designed to hybridize and cleave the target RNA, thus to sterically bind RNA, with a resulting arrest of translation process.

TFOs are synthesized as to bind with a high affinity and specificity to the purine strand in the major groove of homopurine-homopyrimidine sequences in double stranded DNA. They can bind to DNA by parallel or anti-parallel orientation. TFOs directed against the purine-rich tracts of gene promoter regions are able to selectively

reduce the transcription and the expression of target genes, by blocking binding of transcriptional activators and/or formation of initiation complexes. TFOs can be used to mediate site-specific genome modification. Indeed, TFOs are effective by binding as third strands with sequence specificity and the resulting triple helices, or TFO-mutagen complexes, are able to provoke repair and recombination (Faruqi et al, 2000), leading to directed mutagenesis, recombination, and, potentially, gene correction. TFO against p53, *c-myc*, *bcl-2*, HER/neu EGFR, etc have been successfully synthesized (Thomas et al, 1995; Basye et al, 2001; Shen et al, 2003; Re et al, 2004).

4. Effectiveness of antisense approach

i. Experimental validation

The effectiveness of AS-ODN in the reduction of target gene expression has been differently reported in preclinical and clinical studies. *In vitro* studies show that ODNs are effective in the selective inhibition of gene expression (Monia et al, 1996; Eberle et al, 2002; Heere-Ress et al, 2002) and their application in clinical trials is attractive (Crooke, 1993; Hu and Kavanagh, 2003; Stephens and Rivers, 2003). Many experimental studies have been performed with AS-ODNs against several genes and successful chemosensitization and radiosensitization was found in combination treatments both *in vitro* and *in vivo* (Bcl-2/Bcl-xL and TRAIL, MDM2, HER-2, adhesion molecules; Del Bufalo et al, 2003; Rait et al, 2003; Zangemeister-Wittke, 2003; Wang et al, 2003; Tang et al, 2004). Recently, inhibition of *c-myc* and cyclin D1, resulting in a decrease in cell growth, increase of apoptotic index, inhibition of colony formation mediated by a decrease of E2F1 mRNA and protein production has been reported in hepatoma (Simile et al, 2004) and melanoma cells (Eberle et al, 2002). In an androgen-independent human prostate cancer xenograft murine model, an AS-ODN showed inhibition of *c-myc* translation and tumor growth and induction of apoptosis. *In vivo* studies on distribution of *c-myc* AS-ODN locally delivered by gelatin-coated platinum-iridium stents in rabbits indicated an induction of apoptosis in vascular smooth muscle cells, suggesting the efficacy of a local treatment (Zhang et al, 2004).

TFOs directed to regulatory sequences in the *c-myc* gene have been shown to inhibit transcription factor binding and transcription *in vitro* as well as promoter activity and gene expression in HeLa and MCF-7 cells (Postel et al, 1991; Thomas et al, 1995; Kim et al, 1998). Moreover, GT-rich TFOs directed to a sequence near the P2 promoter were particularly effective in inhibiting *c-myc* expression in leukemic and cancer cells (Catapano et al, 2000; McGuffie et al, 2000); daunomycin-conjugated GT-TFOs showed an increased stability of triple-helix and thus a higher activity of the TFO in human prostate (DU145) and breast cancer (MCF-7 and MDA-MB-231) cells (Carbone et al, 2004).

ii. Clinical results

Although ODNs are under clinical investigation in different diseases, the majority of them are exploited

against cancer for which this form of molecular therapeutics seems particularly suitable (Biroccio et al, 2003). ODNs are systemically administered and their toxicities, similar for all compounds, include thrombocytopenia, hypotension, fever and fatigue. AS-ODNs against *c-myc* are currently in phase I study in humans. The lack of toxicity together with the results obtained in a large amount of preclinical results (Iversen et al, 2003; Bayes et al, 2004) support their tentative therapeutic use.

It should be remembered that many other antisense approaches, including for example antisenses against BCL2, XIAP, PKA type I, EGFR, COX-2 inhibitors, gave, alone or in combination with antitumor agents, preclinical encouraging results in patients with advanced solid malignancies (Mani et al, 2003). Indeed this treatment is well tolerated and it is now in Phase III trials on chronic lymphocytic leukaemia, non-small-cell lung cancer, advanced malignant melanoma, multiple myeloma and prostate carcinoma (Hu and Kavanagh, 2003; Kim et al, 2004). Moreover, the effectiveness also of the oral administration of this kind of treatment makes this strategy very promising in cancer therapy (Tortora and Ciardiello, 2003).

iii. Limits of the ODNs approach and attempts to their overcoming

Low physiological stability, intracellular degradation, *in vivo* instability, unfavorable pharmacokinetics (the lack of transfer across cell membranes), low cellular uptake, insufficient nuclear accumulation and accessibility to the target, and the need to deliver AS-ODNs selectively to diseased tissues to maximize their action and to minimize their side effect, together with dissociation of DNA binding, due to changes in DNA or chromatin dynamics,

limit therapeutic applications of AS-ODNs and TFOs (Wagner, 1995) (Figure 4). For this reason, many delivery systems such as viral vectors and liposomes to carry the AS-ODN through the cell membrane and the cytoplasm into the nucleus have been developed (Head et al, 2002). The use of lipid-based delivery systems represents a technological tool for increasing the stability of AS-ODNs *in vivo* (Gutierrez-Puente et al, 1999; Leonetti et al, 2001). The main advantage of liposomes entrapment of AS-ODN is their large carrying capacity, allowing the delivery of a large number of asODN molecules for each binding event. A second advantage is the long circulation longevity of liposome-entrapped drugs in different animal models (Webb et al, 1995; Leonetti et al, 2001) mainly due to a delay of antisense loss by extracellular nucleases.

c-myc-AS-ODN efficiency was increased by delivering the ODN in sterically stabilized liposomes targeted against the disialoganglioside (GD₂) epitope (highly expressed in melanoma cells). Encapsulation of AS-ODNs in GD₂-targeted liposomes can protect non-targeted cells from potential deleterious effects of the AS-ODNs, and simultaneously enhance the toxicity of the molecule toward the target cell population. In these conditions, the down-modulation of *c-myc* determined a reduction of cell proliferation and tumorigenicity and an increased apoptotic rate of human melanoma (Pastorino et al, 2003). To increase the specificity, a selective delivery of immunoliposomes has been obtained with cell surface-directed antibodies grafted on their exteriors (Allen and Moase, 1996) which, however, lose their advantage in the treatment of advanced solid tumors (Allen and Moase, 1996; Lopez De Menezes et al, 1998), likely because the "binding site barrier" restricts the penetration into the tumor (Yuan et al, 1994). Another strategy to increase the

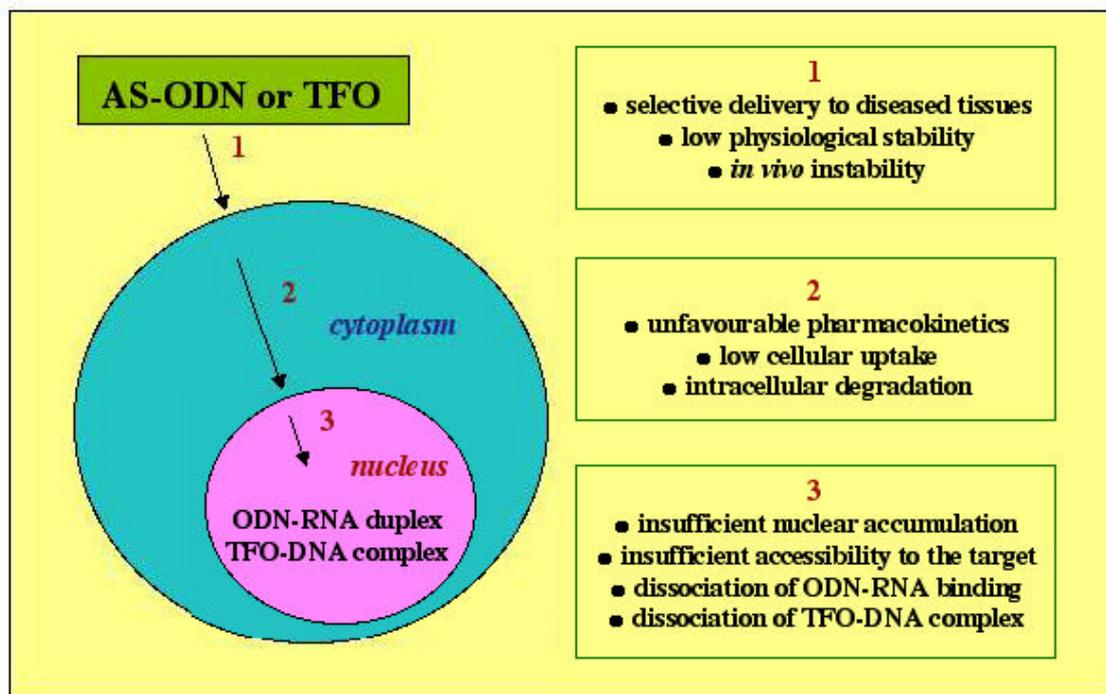


Figure 4. Factors that can limit the use of antisense oligonucleotides (AS-ODN) or triple helix forming oligonucleotides (TFO). Sites 1-3 define where the factors reported in the respective boxes can interfere.

residence time of the oligonucleotides on the target and to increase their stability was to modify ODNs and TFOs as phosphorothioate oligonucleotides, which show a binding affinity similar to that of the phosphodiester oligonucleotide. A marked inhibition of *c-myc* transcription in HeLa cells has been demonstrated (Kim et al, 1998). Advantages in the affinity and the half-life of the binding of TFO to DNA were taken by the daunomycin-conjugated TFO; with this approach *c-myc*-targeted TFO showed a high stability and biological activity in mammary and prostate carcinoma cells (Carbone et al, 2004).

III. Discussion

The oncogene *c-myc* plays essential roles in controlling cell cycle and proliferation, differentiation, tumorigenesis and apoptosis. For its crucial involvement in the development of cancer as well as in driving tumor cells to apoptosis, *c-myc* is a good candidate for the development of strategies aimed at modulating its activity in tumor cells.

In this respect, it is generally assumed that an increased level of *c-myc* could confer a propensity to apoptosis to a tumor cell, which is effective in potentiating the effects of clinical treatments. Even if this pro-apoptotic effect could be cell- and drug-dependent, promising results have been obtained in *c-myc*-overexpressing tumor cells derived from therapy-resistant tumors, such as melanomas and colon carcinomas.

An opposite strategy to face tumor development is the inhibition of the activity of factors that control cell proliferation and transformation, including *c-myc*. This goal is mainly achievable by the use of AS-ODN or TFO. The increasing amount of preclinical data on the effect of AS-ODN to *c-myc* encourages their tentative therapeutic use. However, potential limitation to gene-targeted therapies may exist, e.g. the development of resistant tumor cell populations that lose their sensitivity toward *c-myc* inhibition over time. In addition, since *c-myc* is a factor involved in determining the fate of normal cells and tissues, the side effects of its inactivation have to be considered.

In parallel with the antisense approach, the use of PNA and siRNA could provide an alternative way of down-regulating *c-myc*. The modulation of the functional interaction of *c-Myc* with its partners as well as the development of molecular tools to block the *c-myc* promoter could contribute to improve the anticancer therapy. Further *in vitro* experiments on different cancer cell lines will help in developing clinical trials aimed at obtaining a beneficial up- and down-regulation of *c-myc* in human tumors.

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From left to right: Rosanna Supino and A. Ivana Scovassi