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MYC in breast tumor progression

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Abstract

Breast cancer is the second leading cause of cancer deaths and is the most frequently diagnosed cancer in women of industrialized nations. Breast cancer progression is a multistep process involving genetic and epigenetic alterations that drive normal breast cells into highly malignant derivatives with metastatic potential. *MYC* is a proto-oncogene whose protein product contains a basic helix-loop-helix domain. *MYC* functions as a transcription factor regulating up to 15% of all human genes. *MYC* is regulated at multiple levels, and the protein is a downstream effector of several signaling pathways. In breast cancer cells, *MYC* target genes are involved in cell growth, transformation, angiogenesis and cell-cycle control. *BRCA1* is linked to transcriptional regulation through interaction with *MYC*. Although the relationship between amplification and overexpression is not clearly delineated, *MYC* amplification is significantly correlated with aggressive tumor phenotypes and poor clinical outcomes. *MYC* amplification is emerging as an important predictor of response to HER2-targeted therapies and its role in *BRCA1*-associated breast cancer makes it an important target in basal-like/triple-negative breast cancers.

Keywords

BRCA1; breast cancer; clinical trial; *MYC*; targeted therapy; transcription regulation; tumor progression

The *MYC* protein is a transcription factor containing a basic helix-loop-helix (bHLH) domain that was first identified as the cellular homologue of the viral oncogene of the avian myelocytomatosis virus [1]. Since the amplification and overexpression of the *MYC* gene in human breast cancer were first described in 1986 [2], numerous studies have examined the status of *MYC* in breast cancer. A number of studies consistently found that amplification of *MYC* is correlated with breast tumor progression and a poor clinical outcome.

The cellular functions of *MYC* have been studied extensively and many basic biochemical questions have been solved (reviewed in [3-6]). *MYC* can form a heterodimer with *MAX* and then bind to the E box of its target genes, thus functioning as a transcription factor.

MYC is regulated at multiple levels and it is a downstream effector of several signal pathways, either through transcriptional regulation or protein modification. MYC target genes in breast cancer cells have been implicated in cell growth, transformation, angiogenesis and cell-cycle control. However, the regulation of the physiological functions of MYC and the consequences of its dysfunction in cancer cells remain obscure. While we cannot provide an exhaustive review of all the pathways in which MYC participates, we attempt to summarize the critical role of MYC dysregulation in breast cancer progression.

MYC proteins & E box-binding specificity

MYC is the most extensively studied member of the bHLH proteins. The bHLH proteins form a large superfamily of transcriptional regulators that are found in organisms from yeast to humans and are involved in critical cellular processes. Using the TBLASTN program [7] to search the human genome sequence, Ledent *et al.* identified 125 different human bHLH proteins [8]. Further classification of bHLH proteins can be made on the basis of conservation of amino acids, the presence or absence of additional domains, the protein's target DNA sequence (E box, a consensus hexanucleotide sequence) and binding specificity [8]. The MYC cognate binding partner MYC-associated factor X (MAX) is a bHLH domain protein itself but lacks a transactivation domain. It modulates MYC transcriptional activities through protein-protein interaction. The MYC-MAX heterodimer structure closely resembles that of the MAX homodimer [9]. The heterodimer is stabilized through hydrophobic and polar interactions involving two α -helices, H1 and H2, and the leucine zipper region in the C-terminal region, and most residues in the interaction surface are conserved among the bHLH proteins [9]. Formation of the MYC-MAX heterodimer is required for MYC binding to the E box because artificial MYC homodimers are unable to bind the E box and are defective in biological functions [9]. However, the determining factors for specific binding between the E box and bHLH proteins are not clearly understood. The characteristics of MYC protein binding core DNA have been demonstrated to be more degenerate than originally thought. The E box consensus, CACGTG, was derived from *in vitro* selection experiments [10], and noncanonical E boxes (DNA segments that can bind MYC but do not contain CACGTG) were shown to be associated with MYC-MAX *in vivo* [11]. These E boxes occur frequently in the genome at approximately one in every 1000 bp. If these sites are all functional in physiological conditions, it would make specific transcription regulation impossible. Considering that only a handful of genes have been clearly identified as bona fide MYC transcription targets, E box sequences alone would not be sufficient to determine the selectivity of MYC-E box interactions. Some hints for specificity of MYC-E box recognition come from the nucleotides flanking the E box core sequence. For example, MYC-MAX heterodimers prefer GC residues immediately adjacent to the core E box [9], while MAD-MAX heterodimers have lower selectivity at these sites, possibly due to the different amino acid residues in the basic regions of these proteins [12]. On the other hand, the conserved residues in bHLH proteins involved in the interaction between the loop and nucleotides outside the E box may contribute to target DNA recognition specificity [13]. Cooperative binding at the sites with multiple E boxes or combinatorial binding by binding elements for other transcription factors within a promoter may also affect binding affinity or specificity for subsets of target genes. Methylation of CpG dinucleotide within the core E box may affect DNA binding through modulating the accessibility of target sequences [14]. The abundance of bHLH proteins may contribute to competitive binding of the same target E boxes [15], since it has been suggested that E box-protein interactions in the cell may be a stochastic process [16]. Thus, it is likely that selective MYC binding of a particular E box might depend on the nature of the flanking sequences, the methylation status of the E box and other transcription factors competing for these same sites.

Amplification & overexpression of MYC, pathological features & gene expression profile of breast cancers

MYC may be regulated at multiple levels from *MYC* gene amplification, to overexpression at the mRNA level and protein level, cellular localization and protein stability. The percentage of breast cancer cases that display breast cancer amplification is lower (13% [17], 22% [18]) than the percentage displaying overexpression at the mRNA level (from 22 to 35% [19-21]) or protein level (41.5 [22] to 45% [23]). Thus, the likely mechanisms of overexpression of MYC probably stem from transcriptional, post-transcriptional, translational and post-translational mechanisms. Among literature designed primarily to address MYC status in human breast tumors, studies that evaluated breast tumor progression indicate that *MYC* amplification is detected preferentially in invasive carcinoma or high-grade ductal carcinoma *in situ*, with no amplification observed in associated benign or hyperplastic lesions, suggesting that *MYC* amplification occurs quite late in breast cancer progression [17,18,22-25]. The correlations between MYC status and clinicopathological characteristics are less consistent, even controversial [17,22-34]. This could be largely due to breast tumor heterogeneity and diversity of research methodologies. With regard to the correlation between MYC status and clinical outcome [18,26-28,35], studies that evaluated *MYC* amplification are far more consistent in demonstrating an association with poor outcome than studies investigating mRNA and protein. The protein studies in particular, have been plagued with issues of reproducibility and thresholds for scoring.

MYC amplification or overexpression has been suggested to correlate distinct breast cancer subtypes using DNA microarray analysis. The seminal work by Perou *et al.* demonstrated clearly that mRNA levels of sets of coexpressed genes could be related to specific features of physiological variation, and the tumors could be classified into subtypes distinguished by pervasive differences in their gene expression patterns [36]. These subtypes are luminal A, luminal B, basal, *ERBB2*-overexpressing and normal like; each group has distinct biological features and clinical outcomes [37,38]. The majority (50-67%) of the luminal-type tumors are ductal cancers [39], while the medullary cancers are in the basal-like group [40]. The frequency of *MYC* amplification was threefold higher in the medullary than in the ductal cancer type (seven out of 44, 15.9% vs 60 out of 1077, 5.6%) [41]. The ratio of average *MYC* transcription levels between medullary (n = 22) and ductal (n = 44) breast cancers is 2:1 [40], and that between basal-like (n = 16) and luminal (n = 22) groups is the same (2:1) [42]. Overall, *MYC* is highly expressed in basal-like tumors (seven out of 14; 50%) and also in normal-like cancers (five of 13; 38%), whereas expression is low in luminal (five of 47; 11%) and *ERBB2*-overexpressing (one out of 11; 9%) types [37]. We have previously shown that *MYC* amplification is a frequent event in breast tumors from *BRCA1* germ-line mutation carriers and in sporadic tumors with *BRCA1* inactivation due to *BRCA1* promoter hypermethylation [43,44]. We observed *MYC* amplification in 53% of *BRCA1*-mutated tumors compared with 23% in sporadic tumors. Of the sporadic cases with *MYC* amplification, 57% were *BRCA1*-promoter methylated. In total, *MYC* amplification was found in a significantly higher proportion of tumors with *BRCA1* dysfunction (48 vs 14%, p = 0.0003). In TABLE 1, we have provided a comparison of several important clinicopathological features of breast cancers that have either amplification and overexpression of *MYC* or loss of *BRCA1*. Based on research in our laboratory, we have observed that high-grade, invasive tumors with a high proliferation rate are often found in breast tumor samples with either amplification and/or overexpression of *MYC* or loss of *BRCA1* function. *BRCA1*-associated tumors are also often observed at younger ages of onset and in association with estrogen receptor (ER)- α negativity. Thus, deregulation of *MYC* and loss of *BRCA1* probably contribute to the aggressive features of ER- α -negative breast cancer.

Transcriptional regulation of MYC

MYC is a downstream target of multiple signal transduction pathways, including the Wnt, RAS/RAF/MAPK, JAK/STAT, TGF- β and NF- κ B pathways (reviewed in [45]). The pathways that regulate *MYC* expression relevant to breast cancer progression are discussed later (FIGURE 1).

The Wnt signaling cascade has been well studied with regard to *MYC* regulation in breast tumors. The Wnt signaling pathway is highly conserved in evolution and regulates cell fate, proliferation, differentiation, morphology, migration and apoptosis. The canonical Wnt signal initiates from the binding of the Wnt ligand to the Frizzled receptor, which triggers a cascade that results in β -catenin stabilization and accumulation. The accumulated β -catenin protein translocates to the nucleus, where it forms a transcription complex with the LEF/TCF transcription factor that is inactivated due to binding of transcriptional repressors of the groucho/TLE family. This leads to the activation of the LEF/TCF target genes, including *MYC* [46]. In breast cancers, *MYC* expression levels are strongly correlated with the activation of Wnt signaling components [47]. Ozaki *et al.* performed an immunohistochemical (IHC) analysis of Wnt signaling components using 49 surgically resected primary breast cancer samples. Their data showed that overexpression of β -catenin was strongly correlated with that of *MYC* ($p = 0.0117$), and was significantly correlated with reduced expression of APC ($p = 0.0127$), whose function is involved in degradation of β -catenin. In addition, a positive feedback loop for activation of the *MYC* and Wnt pathways in breast cancer were observed [48]. *MYC* suppressed the Wnt inhibitors DKK1 and SFRP1, and *MYC*-dependent repression of DKK1 and SFRP1 is accompanied by Wnt target gene activation.

Recently, *MYC* expression has been reported to be regulated by the Notch signaling pathway in breast tumors [49,50]. The Notch signaling pathway is a ligand-induced activation cascade of the Notch transmembrane receptor, which is highly conserved in species from worms to humans. Notch receptors play a pivotal role in cell fate decisions, proliferation, differentiation and apoptosis. The Notch signaling pathway relies on two successive proteolyses of Notch receptors. This process creates the intracellular domain of Notch (NIC or N^{IC}), which translocates to the nucleus to form a transcriptional activation complex with the CSL family of DNA binding proteins (CBF1 in humans). *MYC* has recently been identified as a direct transcriptional target of Notch signaling, and Notch1 and *MYC* expression were positively correlated in human breast carcinomas [49]. This was consistent with another study that demonstrated that Notch signaling was activated in breast cancers [50]. Furthermore, they demonstrated that increased Notch signaling was sufficient to transform normal breast epithelial cells and that the mechanism of transformation is most likely through the suppression of apoptosis. While they showed that activation of Notch signaling was not correlated with pathological features, such as the status of ER, progesterone receptor, EGF receptor and HER2/ERBB2, *MYC* status was not addressed. Taken together, it is plausible that *MYC* activation through Notch signaling, as indicated by Klinakis *et al.*, could play a role in breast tumor progression [49].

TGF- β is a ubiquitous cytokine and the TGF- β pathway is involved in cell division, differentiation, adhesion, movement and death. The effectors of this pathway are the Smad family transcription factors. In breast epithelial cells, TGF- β stimulation rapidly induces the accumulation of a Smad transcriptional complex at the *MYC* promoter, leading to repression of *MYC* expression. Formation of this complex is selectively disrupted in breast cancer cells [51]. This transcription complex contains p300 and E2F4, which normally activates *MYC* expression. Replacement of p300 by TGF- β -induced Smad suppresses *MYC* expression [52]. In breast cancers, a decrease in nuclear Smad protein abundance is significantly correlated

with high tumor grade, larger tumor size and hormone receptor negativity [53]. Not surprisingly, these characteristics closely resemble those of breast tumors with amplification and overexpression of *MYC*.

Modulation of *MYC* activity & *MYC* target genes in breast cancer

The *MYC* protein undergoes post-translational modification by phosphorylation, acetylation and ubiquitinylation; these modifications can affect not only *MYC* protein stability but also *MYC* transcriptional activities [45]. The use of cell line models has begun to reveal related mechanisms. Multiple residues of *MYC* are phosphorylated [54], and phosphorylation of Thr58 increases *MYC* protein stability. Sequential phosphorylation of Ser62 coupled with dephosphorylation of Thr58 leads to ubiquitination-mediated degradation. Phosphorylation of *MYC* at Thr58 also regulates its cellular localization [55]. Activation of the estrogen pathway in ER- α -responsive breast cancer cells or elevated phospholipase D activity in ER-negative breast cancer cells affects *MYC* degradation by suppressing phosphorylation of *MYC* at Thr58 [56].

The functional *MYC* protein, which is affected by gene amplification, transcription and post-transcription regulations, would eventually affect its target genes. Recent genome-wide screens using serial analysis of gene expression methods and microarray analyses reveal that *MYC* could target up to approximately 15% of all genes from flies to humans (reviewed in [57]). Some of these genes have been reported to play a role in breast cancer (FIGURE 1).

One of *MYC* target genes, *MTA1* (metastasis-associated protein 1) is essential for the transformation potential of *MYC* [58]. *MTA1* is a component of the multiprotein Mi-2-nucleosome remodeling and deacetylating (NURD) complex, and recruits RNA polymerase II complex to activate downstream targets. Breast cancer amplified sequence (*BCAS3*) is a *MTA1* chromatin target. In one study, *BCAS3* expression was elevated both in mammary tumors from *MTA1* transgenic mice and in 60% of the human breast tumors; this correlated with the coexpression of *MTA1*, as well as with tumor grade and proliferation of primary breast tumor samples. The stimulation of *BCAS3* expression by *MTA1* suggests an important role for the *MTA1-BCAS3* pathway in promoting cancerous phenotypes in breast tumor cells [59].

MYC acts directly upstream of a proliferation-promoting, positively imprinted gene, paternally expressed gene (*PEG*)10 [60]. The *PEG10* gene is paternally expressed/maternally silenced and is located in an imprinted domain on human chromosome 7q21 [61]. *PEG10* expression is dependent on *MYC* activation, and *MYC* protein binds to the E box in the first intron of *PEG10*. In primary breast cancer, *PEG10* and *MYC* protein levels are highly correlated, as demonstrated by IHC analysis [60].

MYC can bind to the E boxes of the *hTERT* promoter region to activate telomerase expression, indicating involvement in cell immortalization. In invasive breast cancer, overexpression of *MYC* is correlated with high *hTERT* expression [24,62]. Hypoxia conditions in the cancer microenvironment rapidly stimulate the transcription activity of HIF-1 α . Activated HIF-1 α regulates a large set of target genes. HIF-1 α can repress *MYC* activated genes by directly displacing *MYC* from the transcription complexes of a set of *MYC* target genes [63]. One such gene is *hTERT*, known to control cell immortalization.

Angiogenesis is an essential step for tumor growth and plays a pivotal role in invasion and metastasis. VEGF is a critical player in regulating angiogenesis and serves as an important prognostic factor in a variety of tumors, including breast tumors. Overexpression of the *VEGF* gene and high blood or tumor levels of VEGF is associated with breast cancer progression [64,65]. An E box in the *VEGF* gene promoter region has been identified, and it

has been shown that MYC can activate *VEGF* expression in colon cancer cell models [66]. VEGF stimulates the growth of breast carcinoma cells, while inhibition of VEGF receptor (VEGFR) function in several human breast carcinomas by anti-VEGFR-neutralizing monoclonal antibodies suppresses tumor growth in xenograph experiments. The authors demonstrate these observations are MYC dependent [67]. Taken together, these data suggest that MYC may activate the VEGF signaling pathway, thus leading to tumor angiogenesis and progression in breast cancer. Several compounds targeting angiogenesis are in clinical trials and have the potential to become the mainstay of breast cancer treatment.

Modulation of MYC activity & *BRCA1*-null or -associated breast cancers

BRCA1 was found to be associated with breast cancer using linkage analysis [68]; inherited mutations in *BRCA1* indicate probabilities of breast cancer by the age of 70 years of 45-87% [69-74]. Recent studies have greatly increased our understanding of *BRCA1* function in both hereditary and sporadic forms of breast cancer (reviewed in [75]). *BRCA1* is involved in a variety of biochemical processes and is an important contributing factor in breast tumor progression. Reduced expression of *BRCA1* is often found in sporadic cases [76-81]. An important *BRCA1* function is to maintain genomic stability by promoting efficient and precise repair of double-strand breaks in response to DNA damage [82].

The *BRCA1* protein has two well-defined domains:

- A RING finger domain, located near the N-terminus of *BRCA1*, which is a zinc-binding domain that mediates protein-protein and protein-DNA interactions [83-85];
- The *BRCA1* C-terminal domain (BRCT), which consists of a tandem duplication of approximately 95 amino acids, loosely conserved in other proteins from a variety of organisms [86,87].

There are two nuclear localization signals in *BRCA1* (503-508, 606-615) which interact with the nuclear transport signal receptor [68,88-90].

BRCA1 has been shown to repress estradiol-responsive ER- α -mediated transcriptional activity [91]. Moreover, *BRCA1* is linked to transcriptional regulation through interaction with a variety of transcriptional components, including p53, CtIP, MYC, the RNA polymerase II holoenzyme complex and the histone deacetylase complex [92-99]. MYC was first identified as *BRCA1* binding partner in a yeast two-hybrid screen with a *BRCA1* fragment (amino acids, 303-1142) as a bait, and the interaction was confirmed *in vivo*. Two regions (183-303, 433-511) of *BRCA1* and the C-terminal region of MYC; (251-439) were critical for the interaction [96]. An efficient *BRCA1*-MYC interaction requires an intact bHLH domain of MYC; however, *BRCA1* does not bind to MAX, an obligate MYC binding partner containing a bHLH domain [96]. *BRCA1* suppresses MYC-mediated transcription activation of the *CDC25A* gene in a promoter activity assay. *BRCA1* also suppresses MYC-Ras cotransformation activity [96]. In another yeast two-hybrid screen using a *BRCA1* fragment (1301-1863) as a bait, Nmi (N-MYC/C-MYC-interacting protein) was identified as a *BRCA1* binding partner [100]. MYC, Nmi and *BRCA1* form a triple complex that represses MYC-activated gene target *hTERT* promoter activity. This repression functions in the presence of functional *BRCA1* but not with the *BRCA1* mutants A1708E and Y1853X [100]. Two regions (298-683, 1301-1863) were identified as being involved in the *BRCA1*-Nmi interaction [100]. Li *et al.* also pointed out *BRCA1* alone did not repress *hTERT* activation [100], while Wang *et al.* demonstrated that *BRCA1* is sufficient to suppress *CDC25A* activation by MYC [96]. The *BRCA1* and MYC complex also transcriptionally repressed psoriasin, a DNA damage-inducible gene, and a *BRCA1* disease-associated mutation (A1708E) abrogated *BRCA1*-mediated repression of psoriasin [101]. These data

indicate that BRCA1 represses MYC transcriptional activity through at least two different distinct pathways by forming different transcriptional complexes. Thus, BRCA1 could serve as a repressor on MYC transcriptional activity. Disruption of this repression, due to inherited *BRCA1* or BRCA1 inactivation in sporadic breast cancer cases, could lead to enhancement of MYC transcriptional potential in breast tumor cells. This over-representation of MYC function could therefore contribute to breast tumor progression.

MYC as a therapeutic target in human breast cancer

The potential of MYC-targeted therapy has been explored in experimental settings using cell line and animal models. However, targeting MYC could become problematic owing to its many roles in normal cellular processes. Moreover, MYC is involved in both proliferation and apoptosis, thus inactivation of MYC could lead to inhibition of tumor cell proliferation and/or increase in apoptosis. Agents targeting MYC have been developed at different biological levels with various techniques, as shown in **FIGURE 2**. These agents may be employed alone or in combination with other current therapies used in the treatment of MYC-associated tumors, as illustrated by Vita and Henriksson [102].

Triplex-forming oligonucleotides (TFOs) recognize and bind to specific duplex DNA sequences, and have been used as selective repressors of gene expression and gene-targeted therapeutics. Reducing *MYC* mRNA expression by introducing TFOs in breast cancer cells has been studied. Downregulation of endogenous *MYC* expression in MCF-7 and MDA-MB-231 breast cancer cells was observed using TFOs targeting the P2 promoter of the human *MYC* gene [103]. TFOs degrade rapidly *in vivo*, therefore modifications to improve TFO stability were undertaken. For example, the linking of the TFOs to daunomycin (a DNA-intercalating agent) resulted in the formation of a DNA triplex with enhanced stability. These TFOs were also effective in cellular uptake. Treatment of SK-BR-3 cells with these modified TFOs reduced MYC expression, and consequently increased the effectiveness of gemcitabine, an anticancer nucleoside analog [104]. Cells treated with combinations of TFOs and gemcitabine showed synergistic reductions of both cell survival and capacity for anchorage-independent growth.

Reduction of *MYC* mRNA level can be achieved by RNAi, a process by which double-stranded RNA silences gene expression, either by inducing the sequence-specific degradation of complementary mRNA or by inhibiting translation. In MCF-7 cells, MYC protein expression was inhibited up to 80% by treatment with a *MYC* antisense oligonucleotide. The cells with depletion of MYC showed decreased cellular proliferation [105]. Introducing siRNA by a plasmid-based polymerase III promoter system against *MYC* into MCF-7 cells markedly reduced MYC protein levels by up to 80%. These treated cells showed a decreased growth rate, inhibited colony formation in soft agar, reduced tumor growth in nude mice and increased apoptosis upon serum withdrawal [106].

The possibility of using decoy oligonucleotides, which can serve as an inhibitor for MYC binding to DNA, to attenuate the effect of overexpressed MYC has been tested [107]. Treatment of double-strand decoy oligonucleotides with the MYC binding consensus site (the cognate E box) in MCF-7 cells decreased cell proliferation in a concentration-dependent manner within 24–48 h, evidently, by reducing MYC transcription activity on its regulated genes containing the cognate E box.

Breast cancer management is multidisciplinary and could include surgery, radiation therapy, chemotherapy, hormone therapy and recently biologic therapy in the adjuvant setting. We retrieved 142 active breast cancer clinical trials from the clinical trials database [201], none was primarily designed to test *MYC* as a target for therapy. However, there are energetic developments of *MYC*-targeted therapies in other disease states. Restenosis is a process of

renarrowing of a coronary artery following a revascularization procedure, and has long been considered a major problem for effective long-term interventional success. Local MYC protein production in the injured coronary artery is a major stimulus and potential cause of restenosis. To inhibit restenosis, an antisense RNA agent (AVI-5126) against *MYC*, developed by AVI BioPharma, is in Phase Ib/II clinical trials for coronary stent restenosis, and another antisense RNA against *MYC*, AVI-4126 (AVI BioPharma) has entered Phase III trials for coronary restenosis [202].

MYC is a marker of proliferation and there is greater interest in evaluating *MYC* amplification as a biomarker to predict response in clinical trials evaluating trastuzumab (a humanized monoclonal antibody that acts on the HER2/ERBB2) and lapatinib (a tyrosine kinase inhibitor) in breast cancer (NCT00486668 and NCT00542451) since MYC is speculated to potentiate apoptosis. Preliminary results from the National Surgical Adjuvant Breast and Bowel adjuvant trastuzumab breast cancer trial (North Central Cancer Treatment Group-N9831) suggest that HER2-positive breast cancer patients with *MYC* amplification derived more benefit from trastuzumab than those patients without *MYC* amplification [108].

Expert commentary

Detailed analyses of *MYC* amplification and *MYC* overexpression in breast cancer samples implicate *MYC* in breast tumor progression and clinical outcome. However, the many methods utilized in the determination of gene copy number, and both RNA and protein expression levels have led to conflict in the interpretation of such studies. While gene amplification studies show consistent associations with poor outcome, the assessments of MYC protein expression by IHC provide variable results depending on the antibody, testing protocol and scoring system used. Addressed less often, is the unexpectedly loose correlation between gene amplification and overexpression of mRNA and protein. Controversial results are largely caused by methodology and evaluation protocols. It appears that valid and reproducible methods need to be improved in order to accurately measure MYC status in primary tissue samples. Since the MYC protein is subject to post-translation modification and its function is greatly affected by these modifications, it would be ideal to monitor the functional form of MYC.

Five-year view

Gene regulatory elements, such as the E box and noncanonical E box sequences, have been found in the genome at a high frequency (almost one in 1000 nucleotides) due to recent progress in genome sequence analysis. However, their physiological role is just starting to be understood. It appears that the transcriptional machinery is complicated and in a dynamic state, specifically with regard to the binding of transcriptional factors to a core component. The E box has been clearly demonstrated to be differentially associated with HIF-1 α and MYC for a set of genes under specific cell growth conditions. There are 125 proteins predicted to contain a bHLH domain, many of which could have the ability to bind to E boxes. Not surprisingly, some of the proteins, such as Twist and HIF-1 α , have been shown to be involved in MYC-related breast cancer progression. Considering the network among MYC, its protein partners and DNA binding targets, the next step would be to determine the exact components of the MYC transcription complex and its function in breast tumor progression. These results could provide the basis for developing MYC-target based therapies in cancer treatments.

Key Issues

- Breast cancer progression is a multistep process involving genetic and epigenetic alterations.
- *MYC* amplification correlates with breast tumor progression.
- *MYC* is highly expressed in basal-like tumors.
- Wnt, Notch and TGF- β signaling pathways play important roles in regulating *MYC* expression in breast cancers.
- BRCA1 can repress *MYC* transcription activity.

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Figure 1. Signal pathways modulating *MYC* expression and the potential cellular processes involved in breast tumor progression controlled by *MYC*

MYC gene transcription is activated by a variety of growth factors (Wnt and Notch are shown), yet repressed by other growth factors, such as TGF- β . The MYC-MAX dimer is required for MYC transcription activation function, and MYC-MAX interaction is modulated by other basic helix-loop-helix proteins, such as MAD. Functional MYC activates the expression of key factors involved in breast tumor progression.

Figure 2. Possible biological process that can be used for *MYC*-targeted therapy

Agents designed to reduce *MYC* gene expression at the levels of transcription and mRNA stability are being tested. *MYC*-MAX interaction, *MYC* protein degradation and interference with transcription of *MYC*-activated genes are promising fields for future drug design.

*Less explored area of *MYC*-targeted therapy is *MYC* gene amplification.

Table 1

Clinicopathological features of amplification and overexpression of MYC and BRCA1-associated breast tumors.

Feature	MYC-amplification or overexpression	BRCA1-loss
Grade	High	High
Histology	Invasive tumor	Invasive tumor
Proliferation rate	High	High
Age	NA	Younger age
ER- α status	NA	Negative
Data are derived from works of our laboratory.		
NA: Not associated.		