

MYB Is Essential for Mammary Tumorigenesis

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Abstract

MYB oncogene upregulation is associated with estrogen receptor (ER)-positive breast cancer, but disease requirements for MYB function in vivo have not been explored. In this study, we provide evidence of a critical requirement for MYB functions in models of human and murine breast cancer. In human breast cancer, we found that MYB expression was critical for tumor cell growth both in vitro and in vivo in xenograft settings. In transgenic knockout mice, tissue-specific deletion of the murine MYB gene caused a transient defect in mammary gland development that was reflected in delayed ductal branching and defective apical bud formation. In mouse mammary tumor virus (MMTV)-NEU mice where tumors are initiated by activation of HER2, MYB deletion was sufficient to abolish tumor formation. In the more aggressive MMTV-PyMT model system, MYB deletion delayed tumorigenesis significantly. Together, the findings in these transgenic knockout models implied that MYB was critical during an early window in mammary development when it was essential for tumor initiation, even though MYB loss did not exert a lasting impact upon normal mammary function. Two important MYB-target genes that promote cell survival, BCL2 and GRP78/BIP, were each elevated compared with nontransformed mammary epithelial cells, thereby promoting survival as confirmed in colony formation assays in vitro. Taken together, our findings establish a role for MYB at the hub of ER- and HER2-dependent pathways in mammary carcinogenesis.

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Introduction

Breast cancer is heterogeneous in its genetic makeup and multifaceted in etiology (1, 2). In addition to estrogen receptor α (ERα), high expression of the receptor tyrosine kinase HER2 and the status of progesterone receptors are prognostic and therapeutic guides in breast cancers management. Although the majority of breast cancers express one or more of these receptors, some are “triple-negative” (3); this subtype is often more aggressive and does not respond to conventional adjuvant therapies that target the above-mentioned receptors (4, 5). With such phenotypic diversity, the prospect of identifying molecular points of convergence that drive breast cancer may seem small although nonetheless enticing.

The appeal of considering transcription factors in the context of breast cancer is that they are often downstream of multiple signaling pathways and their target genes can fall into promalignancy categories that cooperate in tumorigenesis. We have focused on the transcription factor, MYB (6) for which target genes in myeloid cells (7) and breast cancer (8) have been reported. A central observation that underpins our attention on MYB is that it is frequently overexpressed in breast cancer (9). We reported the direct regulation of MYB transcriptional elongation by ERα (6), that MYB is essential for proliferation of breast cancer cell lines (6), and that it suppresses differentiation and apoptosis (10). As ~70% of breast cancers are ERα-positive, this work addressed a significant breast cancer category. Nevertheless, we report here that MYB is also evident in some HER-positive breast cancer in the absence of ERα. At the other end of the spectrum are BRCA1-mutant breast cancer (11), representing only a small percentage of all cases. Intriguingly, however, 29% of BRCA1-mutant breast cancers have amplified MYB (12), further suggesting that MYB has broad relevance to breast cancer.

To specifically address the role of MYB in mammary cancer, we have now employed 2 transgenic mammary-specific tumor models. Both depend upon the mouse mammary tumor virus (MMTV) promoter to drive either the wild-type (wt) rat NEU gene (13) or the Polyoma middle T-antigen viral gene (PyMT; ref. 14). These models have provided many important insights into mammary cancer progression. We report here that with the specific ablation of MYB expression in the mammary gland, neither transgenic model progresses normally to form tumors.
In vitro studies show that MYB protects mammary epithelial cells from apoptosis, allows colony formation in soft agar, and drives cancer-associated gene expression. Collectively, these data establish a central and pervasive role for MYB in mammary carcinogenesis.

Materials and Methods

Xenografts

A total of 1 × 10^6 ZR-75-1 cells [stably expressing either inducible MYB short hairpin RNA (shRNA) or scrambled shRNA and constitutive enhanced (eGFP)] under the control of EF-1α promoter (6) in 50 μL complete medium were mixed with 50 μL Matrigel (BD Biosciences) and injected into the vicinity of the mammary gland of 6- to 8-week-old female nonobese diabetic (NOD)/severe combined immunodeficient (SCID) mice. Estradiol pellets (0.72 mg/pellet, 60-day release; Innovative Research of America) were implanted 3 days before cell injection. Mice were imaged 2 weeks later with an Imaging Station (In Vivo FX; Eastman Kodak Company) for eGFP expression. After confirmation of the presence of tumors, mice were divided into 2 groups with equal-sized tumors. To induce shRNA, mice were given chow supplemented with 600 mg/kg doxycycline (specialty feeds). Tumor growth was measured by eGFP intensity at fortnight intervals.

Mice

Mice with IoxP sites introduced between MYB introns 2 and 6 were kindly provided by Jon Frampton (Birmingham, England; ref. 15). These mice were then crossed with MMTV-Cre (ref. 16; C57Bl/6) and/or MMTV-NEU (wt rat Neu; F1—FVB/N; C57Bl/6—mixed background littermates; 6 generations on C57Bl/6). F1 backcross litters were generated with matched genotypes [MMTV-Cre; MMTV-NEU; MYB^f/f; or MMTV-PyMT MYB^f/f; MYB^f/f; MMTV-Cre (C57Bl/6)]. All MMTV-NEU females experienced 2 pregnancies. Animals were bred and maintained in a pathogen-free environment and with approval by the Peter MacCallum Cancer Centre Animal Ethics Committee.

Tumor microarrays and immunohistochemistry

Tumors (n = 69) from women without a family history of breast cancer were collected with consent from a single-site State Pathology Centre. These were processed for immunohistochemistry (IHC) for HER2 (4B5; rabbit monoclonal Ventana), ERα (SP1; rabbit monoclonal Ventana), and pan-MYB (Mab1.1; Upstate Biotech) as described (17), detected by horseradish-peroxidase conjugated anti-mouse or anti-rabbit secondary antibodies (DAKO), visualized with 3,3’-diaminobenzidine tetrachloride (DAKO), scanned (Aperio slide scanner) and scored blind by 2 observers. Mouse tissues were fixed in Methacan. Proliferating cell nuclear antigen (PCNA) was detected with mouse antibody PC-10 (Santa Cruz).

Cell culture and expression

Murine cell lines 67NR, 66cl4, 4T1, and 4T1.2 were derived from a spontaneous mammary tumor (ref. 18; verified by array comparative genomic hybridization analysis, all 3 lines are essentially identical to the original 4T1 cell line; unpublished data). ZR-75-1 cells [purchased from American Type Culture Collection (ATCC)] were reconfirmed by short tandem repeat polymorphism (STRP) analysis in the past month (19). TA93 and PyMT cell lines were established from MMTV-NEU and MMTV-PyMT tumors, respectively, by the authors of this article (unpublished data). NMuMG cells were originally purchased from ATCC. Primary tumors were collected from MMTV-NEU; PyMT or Wnt-1 mice. Retroviral vectors pRuNeo-MYB (murine MYB, full length) and pRuNeo-control were used to generate retroviral supernatants for stable transductions (20). Western analysis was conducted as previously described (21, 22).

Anchorage-independent growth

NMuMG MYB transduced and control cells were plated in 0.35% agarose in 6-well plates (15,000 cells/well). Medium was added every 4 to 5 days and colonies photographed at day 21.

Mammary gland analysis

Mammary glands whole mounts were spread on a glass slide before fixing in Carnoy fixative. Glands were stained in 0.2% carmine and 0.5% aluminum sulfate overnight. Subsequent analysis was carried out using Metamorph image software.

PCR

PCR was used to amplify genomic DNA for the detection of MYB, Cre, and NEU genes (primers; see Supplementary Table S1). For real-time quantitative reverse transcriptase PCR (qRT-PCR) analyses, primers were designed using Primer Express software (Supplementary Table S2) and transcripts were amplified using a Prism 7000 Sequence Detection system (ABI).

Fluorescence-activated cell sorting

Fluorescence-activated cell sorting (FACS) was used to detect apoptotic cells by Annexin V antibody binding (BD Pharmagens) and 7-amino-actinomycin D (7AAD) staining. Data were analyzed using the fetal calf serum (FCS) express program (De Novo software).

Statistical analysis

Data were subject to analysis using Graph-Pad Prism5.

Results

MYB knockdown in vivo

Having established that MYB is required for proliferation of human ER-positive breast cancer cells in vitro (6), we asked here whether persistent expression was required for established tumors. ERα-positive ZR-75-1 cells with inducible shRNA directed against MYB (6) were generated with an eGFP expression module to allow in vivo xenograft imaging. Tumors were established orthotopically for 2 weeks before shRNA expression was induced with doxycycline (Fig. 1A); subsequent monitoring (Fig. 1B and C) showed that MYB expression is required for ongoing tumor growth. Similar data were obtained with MCF-7 cells (data not shown). These data indicate that not only is MYB expression a feature of human ER-positive breast cancer cells in vitro but that sustained MYB expression is required for tumor growth.
MYB in HER2-positive/ERα-positive breast tumors

MYB expression is a consistent feature of ERα-positive breast cancer (reviewed in ref. 9). We have shown above that it is required for growth in vivo. By evaluating breast cancers from 69 women without a family history of breast cancer in a tissue microarray (TMA) format, we found most expressed MYB (64/69). Furthermore, coincident expression of ERα-positive and MYB (56/64; Supplementary Fig. S1A–C) is consistent with previous reports based mostly on cell lines and mRNA analyses (6, 10, 23). Importantly, Mab1.1 also detects other MYB family members and to confirm that the signal observed with the TMAs was MYB, we employed another antibody Mab5.1 (22) that we determined to be MYB-specific (data not shown). Both Mab1.1 and 5.1 gave comparable IHC (data not shown).

Our IHC observations were consistent with the mRNA profiling of breast cancers where MYB tracks with ERα expression (reviewed in ref. 9); however, the status of MYB in HER-positive tumors has been less clear. In TMAs we identified 13 tumors with robust HER2 expression, 12 of which were also MYB-positive. Of these, we focused on the 4 that were MYB-positive and HER2-positive but ERα-negative. MYB expression was identified associated with tumor cells in the HER2-positive (ERα-negative) field and in an apparently normal ductal region where cells were ERα-positive, MYB-positive, but HER2-negative (Fig. 2B and C). From these examples, it is apparent that MYB expression can occur without ERα driving MYB transcription and pathways that are HER2-dependent may also be associated with MYB expression.

MYB expression in mouse mammary tumors and NEU-induced tumorigenesis

Here, we extended previous studies with human breast cancer cell lines to examine MYB in mouse mammary tumors. A range of spontaneously arising clones of different metastatic capacity (4T1.2, 66CL4, and 67NR; refs. 24, 25), cell lines from MMTV-NEU (TA93) and MMTV-PyMT (PyMT) tumors and primary tumors were compared with an immortalized mammary epithelial cell line (NMuMG; ref. 26) or to normal mammary gland. MYB mRNA and protein were found to be higher in tumor cell lines and tumors (MMTV-Her2/Neu; MMTV-PyMT; and MMTV-Wnt-1) than in adult mammary gland (Supplementary Fig. S2A–C). By IHC, we also showed MYB nuclear staining in MMTV-NEU tumors (Fig. 3A) and consistently elevated MYB mRNA in tumors resected from these mice (Fig. 3B).

MMTV-NEU mice were then crossed with MYBf/f × MMTV-Cre mice. MYBf/f mice have been described previously (15) and have been used by us to show that MYB is required for colon epithelial homeostasis (27). However, they have not been employed to examine tumorigenesis in epithelial tissues. To ensure both efficient tumorigenesis and Cre-mediated recombination, all females were subjected to 2 rounds of pregnancy and lactation. All MMTV-NEU:MYBf/f females developed at least 1 mammary tumor between 300 to 500 days, which is consistent...
with other reports (13). By contrast, at 500 days no MMTV-NEU; MMTV-CreMYBf/f females had palpable tumors. Later, 3 mice in this cohort of 14 mice developed tumors (Fig. 3C), whereas the remaining mice died of old age, tumor free. These late-onset tumors from knockout (KO) mice retained MYB expression albeit at lower levels (but higher than normal mammary glands; Fig. 3D), indicating that MYB was not completely deleted in these cases. To ensure that the expression of the oncogenic

Figure 2. MYB and HER2 coexpression without ERαx. A, serial sections of 4 breast tumor samples (i–iv) are represented where MYB is evident in the regions of relatively robust HER2 staining but where ERαx is essentially absent. Brown arrows denote regions that coexpress MYB and HER2 but are ERαx-negative (white arrow). B, in sample (iv) a region that is histologically normal, HER2-negative and ERαx triple positive are also positive for MYB (C).

Figure 3. Mammary tumors show elevated MYB expression and MMTV-NEU tumor-prone mice lacking MYB in the mammary gland display prolonged survival. A, MYB protein IHC in an MMTV-NEU tumor (ii and iv). IgG control (i and iii); scale bar = 50 μm. B, MYB levels were determined in 4 tumors (T1–4) harvested from MMTV-NEU mice compared with normal mammary glands (Mg1–4). C, Kaplan–Meier analysis of tumor-free survival in female MMTV-NEU/MYBf/f (n = 28) and MMTV-NEU/MYBf/f;MMTV-Cre (n = 14) mice. D, analysis of the 3 tumors (T1a–3a) that developed in MMTV-NEU/MYBf/f;MMTV-Cre mice revealed modest MYB expression indicating incomplete MYB deletion. (****, *P = 0.0001; scale bar = 50 μm.)
**MYB expression is required for timely MMTV-PyMT tumorigenesis**

Given that very few *MMTV-NEU;MMTV-Cre* mice developed mammary tumors and that the incidence of tumor formation in the *MMTV-NEU;MYBfl/fl* control mice was 100% (28/28), MYB expression is clearly shown to be a limiting factor in this model. The *MMTV-NEU* model takes almost 1 year before mice begin to develop mammary tumors. By contrast, the viral oncogene PyMT leads to a rapid onset of multifocal mammary tumors beginning at 10 weeks (14). Initially, we established that primary tumors from *MMTV-PyMT* females consistently expressed MYB (Supplementary Fig. S2B and Fig. 4A and B). We then introduced the *MYB* locus and the *MMTV-Cre* transgene. The deletion of 1 *MYB* allele led to a significant delay in time to selection of tumor bearing *MMTV-PyMT; MMTV-Cre MYBfl/+* females, whereas deletion of both alleles (*MMTV-PyMT; MMTV-Cre MYBfl/fl*) markedly impeded tumor development and doubled life expectancy (Fig. 4C).

**Figure 4.** MYB KO delays MMTV-PyMT tumorigenesis. A, MYB protein detected by IHC (i) (IgG antibody control) and (B) mRNA expression in *MMTV-PyMT; MYBfl/fl* mammary tumors (T1–4) and unaffected mammary glands (Mg1–4). C, tumor-free survival of mice bearing the *MMTV-PyMTfl/fl* transgene: mice were assigned to 3 groups; 13 *MMTV-PyMT; MYBfl/fl* (wt), 14 *MMTV-PyMT; MYBfl/fl; MMTV-Cre* (heterozygous for MYB), and 15 *MMTV-PyMT; MYBfl/fl; MMTV-Cre* (KO). (**, *P* = 0.0001; scale bar = 50 μm.)

Approximately one quarter of this cohort of mice was still disease free at 700 days, which is remarkable given that the *PyMT* transgene typically leads to highly aggressive and multifocal mammary carcinogenesis. By contrast, in *MMTV-NEU* mice where tumors were rarely detected, at least not until 500 days in KO mice *MMTV-PyMT* females did develop tumors. To confirm that the basis for tumor delay was not due to an effect of KO on PyMT expression, PyMT mRNA levels were evaluated in tumors from wt and KO mice. Although levels of MYB varied, perhaps indicative of tumor heterogeneity and KO, it was very clear that PyMT transgene expression was consistently high regardless of MYB level (Supplementary Fig. S3B). Interestingly, tumors were isolated that had either very low or robust MYB mRNA expression on a background KO, indicating that the cells that gave rise to some of these tumors had not been subject to MYB deletion as might have been expected in the presence of MMTV-Cre. Such mice were reconfirmed to be of the designated genotypes (data not shown). This suggests that PyMT can transform both MYB-negative and MYB-positive mammary target cells more rapidly than in cells expressing the NEU transgene.

**C(-)MYB and A-MYB in developing mammary glands**

In view of the inhibition or delay of mammary tumorigenesis in mice on a KO background, perhaps MYB loss might lead to the ablation of cells that are targeted for tumorigenesis or perhaps even a general loss of mammary epithelial cells. Mammary gland development has been characterized extensively (28), but a role for MYB in this tissue has not been examined as global MYB KO mice die at E14.5 (29). By contrast, A-MYB KO mice are viable but have defects in mammary gland function (30). We compared the expression patterns of MYB and A-MYB during mammary gland development compared with housekeeping gene *GAPDH* (Fig. 5A; and *Cytokeratin-18*; Supplementary Fig. S4) expression. MYB expression was initiated earlier and at higher levels relative to A-MYB in virgin glands before it declined. Notably, luminal MYB is most evident in virgin glands (Fig. 5B–D), and proportionally more cells were MYB-positive than those actively engaged in the cell cycle (PCNA-positive) or those that were ERα-positive (Supplementary Fig. S5).

To test whether MYB loss might be of functional consequence, we examined the mammary glands of MYBfl/fl mice crossed with MMTV-Cre mice. Figure 5E–G reveals delayed ductal branching and terminal end bud (TEB) formation in KO glands. Importantly, female mice carrying the mammary-specific MYB deletion became pregnant, give birth to litters of normal size, and suckle their pups to normal body weights (data not shown), indicating that the *MMTV-Cre* line employed by us was not causing lactation defects (31). The presence and ultimate resolution of mammary gland developmental defects in the KO mice were in marked contrast to other KO mice such as *CyclinD1, SRC*, and *A-MYB* that showed a lasting negative effect on mammary gland development (32, 33). Therefore, it is reasonable to speculate that the delayed mammary development may be in part responsible for impeding a key event in the initiation of mammary carcinogenesis that is MYB-dependent.

**MMTV-NEU** transgene was not under the transcriptional influence of MYB in normal mammary tissue RNA (DNAse-treated) was prepared from adult female KO and wt mammary glands and subjected to qRT-PCR whereby rat NEU cDNA expression was found to be relatively robust and unaffected by the loss of *MYB* (Supplementary Fig. S3A).
MYB induces anchorage-independent growth and enhances cell survival

We next sought to establish how MYB might contribute to mammary cell transformation. We transduced NMuMG cells (26) with a MYB retrovirus. Normally, NMuMG cells grow attached to the substratum in 2-dimensional (2D) tissue culture but show very little growth in agarose. By contrast, MYB-transduced cells displayed increased anchorage-independent growth (Fig. 6A and B). Under suboptimal growth conditions (FCS 0.1%) NMuMG cells attach but show relatively poor growth in 2D culture, whereas MYB-transduced cell displayed robust growth (Fig. 6C) associated with enhanced survival as assessed by Annexin V staining (Fig. 6D). The expression of 2 MYB-target genes associated with cell survival that are overexpressed and regulated by MYB in colorectal cancer and breast cancer, Grp78/BIP, and BCL-2 (10) were elevated (Fig. 6E and F). These data extend our previous findings that MYB suppresses differentiation and apoptosis of human breast cancer cell lines (10).

Discussion

The role for MYB in hematopoietic malignancies has overshadowed its important role in epithelial cancers (9). RNA profiling studies collectively support the view that high MYB tracks with ERα-positive breast cancer compared with most other epithelial cancers with the notable exception of colorectal cancers where MYB is not only overexpressed but also of prognostic importance (34). Accordingly, our focus has been on ERα-positive breast cancer as this is the predominant subtype and we have made an important functional leap to show here the requirement for sustained MYB expression in established breast cancer xenografts. We also showed that HER2-positive breast cancers are MYB-positive.
Together with our IHC data, it seems that obviously in patients with luminal-type breast cancer (36) associated with improved survival in another study most A or B, or normal-like breast cancer. High MYB with high survival, particularly in patients with ER

Promoter (13). Here, we have shown that MYB is required for NEU-driven tumorigenesis in these mice. Previously, we focused our attention on MYB in the context of ERα-positive breast cancer, but now we have to consider MYB in the broader context of HER2-positive breast cancer. Thus, this role in HER-positive breast cancer which is the next most clinically tractable group to treat after ERα-positive breast cancer further raises the importance of MYB more generally in breast cancer. Indeed, recent analyses of breast cancer expression profiling indicates that MYB is associated with high survival, particularly in patients with ERα-positive tumors (35) but that MYB did not track specifically with identified molecular subtypes such as basal, HER2, luminal A or B, or normal-like breast cancer. High MYB was also associated with improved survival in another study most obviously in patients with luminal-type breast cancer (36). Together with our IHC data, it seems that MYB expression arises in a range of breast cancer subtypes. However, in contrast to an earlier pioneering report by Guerin and colleagues (23) that emphasized an inverse relationship between HER2 and MYB where they still observed coexpression in 56% (24/56) and discordant expression in 77% (53/69) of noninflammatory breast cancer, we found by IHC that 92% of HER-positive breast cancer coexpressed MYB (12/13). ERα expression was present in 9 of 13 HER-positive breast cancers that we examined; however, MYB and HER2 can be coexpressed in the absence of ERα (4/13 HER2+ve tumors).

This relationship between MYB and mammary carcinogenesis is not without complexities as the MMTV-PyMT mouse data suggested. In these mice, we found that although MYB was required for the normal kinetics of tumorigenesis its ablation did not stop tumor formation in 75% of female mice and indeed the tumors that arose were both MYB-positive and low. Conversely, in the absence of this genetic selection most tumors that arose in MMTV-PyMT mice had detectable MYB expression but the levels were variable. These data suggested that MYB was required for the timely development of mammary tumors but was not ultimately essential for their progression. Certainly, we and others have reported that MYB is not in all breast cancer cell lines; MDA-MB-231 is a notable ERα-positive and MYB-negative cell line (6) and 15 of 133 breast cancer were MYB-positive in the TMAs (Supplementary Fig. S1). However, the link between these models might be that mammary tumorigenesis may proceed through an ERα-dependent phase where PyMT but not NEU expression may more readily progress in the absence of MYB.

In vitro studies were used to show that MYB modifies the growth of immortalized mammary cells and activates genes associated with cell survival. To probe the functional role of MYB during mammary gland tumorigenesis, the normal
mammary epithelial cell line NMuMG expressing full-length MYB was assessed for the expression of established MYB target genes, Gpr78 and BCL-2, which were found to be consistently upregulated. NMuMG cells transduced with MYB showed enhanced proliferation and reduced apoptosis likely due to the elevated expression of these prosurvival genes (37). However, elevated MYB did not alter cell migration as measured by the scratch assay (data not shown).

Although many studies have documented MYB-target genes (9) less is known about the mechanisms that regulate MYB expression itself. Certainly, the MYB promoter is subject to regulation by the phosphoinositide 3 kinase and AKT pathway in T cells (38) and can be autoregulated by MYB itself (39, 40). Beyond promoter activation, a principle mechanism of MYB regulation is the ability of exon-1 transcripts to be extended by transcriptional elongation (41–44) and in the case of colon cancer, we have shown that mutations in a region that regulates this process are relatively common in the human gene (9). However, similar mutations were not detected in human breast cancer or cell lines (42); rather, we found that the ERα when bound by estradiol controlled elongation through this region (6). We have not as yet established the same relationship between MYB transcription and ERα in mouse mammary cells perhaps because most mouse mammary cancer lines are typically ERα low or negative by the time they are in culture. It would seem that there is a strong case that most tumors also progress through an ERα-positive stage. This has been shown most convincingly with MMTV-PyMT mice that develop adenoma/mammary intraepithelial neoplasia that closely resembles the ductal epithelial breast hyperplasia observed in humans. These lesions progress to mimic ductal carcinoma in situ. The primary tumors are typically 30% to 40% ERα-positive, and this may increase to 80%. As the carcinoma progresses, ERα-positive cells emerge becoming the predominant category. Approximately 30% of early carcinoma may have 70% to 80% partial remission positive nuclei, but this expression also disappears in later stages of carcinoma progression (45). Similarly, measures that modify ERα status and/or function can delay or block tumorigenesis in the MMTV-NEU model (46).

IHC studies revealed MYB and ERα-positive cells in the ductal epithelium of mouse mammary glands, but as there are more MYB-positive, than ERα-positive cells (Supplementary Fig. S5), the question remains as to whether the former is regulated by the latter. However, it is clear that without MYB, tumorigenesis is markedly inhibited. Similarly, the relationship between NEU and MYB regulation has been intriguing. It is notable that NEU expression is evident in both the normal epithelia and early-stage tumors and increases with progression, whereas there is a commensurate reduction that tracks with ductal and TEB maturation. At the protein level, NEU increases 3- to 5-fold in tumors compared with adenoma/mammary intraepithelial neoplasia. Although relatively high during mammary gland development, NEU expression declines as gland maturation is nearly complete (45). Whether these events occur in a parallel fashion in human breast development and breast cancer is less clear. Similarly, it is unclear whether HER2 can drive MYB expression in breast epithelial cells and if so, this regulation may have to take into account the observation that MYB has been reported to repress HER2 transcription in non–breast cancer cells (47).

The mouse studies used here show that established human breast cancer xenografts do not advance when MYB is knocked down using shRNA, and that NEU mammary tumors are rarely formed without MYB. Taken together with our previous observations made on the direct role of MYB human breast cancer cell growth and progression (10), we have now provided evidence across a spectrum of models that argues for a role for MYB in mammary carcinogenesis. Finally, these findings may contribute to the understanding and treatment of human breast cancer. For example, the KO data have implications for the etiologic timing of breast cancer initiation in young women (48), whereas the expression pattern and essential role of MYB in multiple forms of mammary carcinogenesis strongly encourage the pursuit of therapeutic approaches that directly or indirectly target MYB (10, 49).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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