Review

Key signalling nodes in mammary gland development and cancer
Myc

Nancy E Hynes and Tina Stoelzle

Friedrich Miescher Institute for Biomedical Research, Maulbeerstraße 66, CH-4058 Basel, Switzerland

Corresponding author: Nancy E Hynes, nancy.hynes@fmi.ch

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Abstract

Myc has been intensely studied since its discovery more than 25 years ago. Insight has been gained into Myc's function in normal physiology, where its role appears to be organ specific, and in cancer where many mechanisms contribute to aberrant Myc expression. Numerous signals and pathways converge on Myc, which in turn acts on a continuously growing number of identified targets, via transcriptional and nontranscriptional mechanisms. This review will concentrate on Myc as a signaling mediator in the mammary gland, discussing its regulation and function during normal development, as well as its activation and roles in breast cancer.

Introduction

Since the early 1980s, numerous investigations have focused on c-Myc to explore its role in normal organ physiology, as well as in tumor biology [1,2]. The focus of the present review, c-Myc (hereafter referred to as Myc), is the cellular homolog of the avian retroviral oncogene v-*myc* and, together with N-*myc* and L-*myc*, comprises the family of *myc* proto-oncogenes. The half-lives of Myc mRNA and protein are short, allowing for tight and rapid regulation of Myc levels, which occurs via numerous transcription factors (TFs) and signaling pathways. Proteins that directly bind the promoter or indirectly influence promoter activity have been reviewed recently [3]. To provide some insight into the complexity of Myc regulation, we will mention a few of the factors and pathways that impact on its expression, many of which were shown to be essential during mammary gland development [4].

The *myc* promoter contains TF binding sites for Myc (autosuppression), estrogen receptor (ER) alpha, T-cell factor (TCF) 4, Notch/C promoter-binding factor 1 (Cbf1), E2F, Fos/Jun, signal transducer and activator of transcription (Stat) 3, NF-κB, Smads and others. TFs that occupy or regulate the *myc* promoter without specific binding sites include p53, CCAAT/enhancer binding protein beta, and Stat5. Moreover, numerous signaling pathways that are frequently deregulated in human cancer influence *myc* expression; for example, rat sarcoma (Ras)/extracellular signal-related kinase (Erk) and phosphoinositide 3-kinase (PI3K)/serine/threonine kinase Akt (Akt). Post-translational modifications of Myc include phosphorylation, ubiquitinylation and acetylation, and their effects on Myc activity have been reviewed [5].

The Myc protein is a basic helix–loop–helix TF that must heterodimerize with the abundantly expressed Max to regulate transcription. Myc–Max dimers bind to hexameric DNA sequences (E-box) and activate transcription by recruiting multiple coactivators [1]. In contrast, when dimerized with basic helix–loop–helix proteins such as Mad or Mnt, Max binds to E-boxes but represses transcription. Myc can also act as a transcriptional repressor via different mechanisms, often involving interaction with Miz1 (for reviews of Myc transcriptional activity, see [1,6]). It is now well accepted that Myc acts as a relatively weak activator of RNA polymerase II-driven transcription for a large set of target genes, thereby affecting cell cycle, cell growth and metabolism, cell death, adhesion, angiogenesis and other functions (to date, almost 1,700 targets on Myc Cancer Gene [7,8]). Furthermore, Myc affects RNA polymerase I- and III-mediated transcription [1], thus regulating ribosome biogenesis and translation. Nontranscriptional roles for Myc in DNA replication and in translation have also been reported recently [9].

Considering the large number of receptors, hormones, paracrine factors and other signaling molecules that can

Akt = serine/threonine kinase Akt; APC = adenomatosis polyposis coli; Cav1 = Caveolin1; Cbf1 = C promoter-binding factor 1; Cdk = cyclin-dependent kinase; ER = estrogen receptor; Erk = extracellular signal-related kinase; K14 = Keratin14; MMTV = mouse mammary tumor virus; MTB/TOM = MMTV-rtTA/TetO-MYC; NF = nuclear factor; N^{IC} = Notch intracellular domain; Nrg3 = Neuregulin3; PI3K = phosphoinositide 3-kinase; Ras = rat sarcoma; SC = stem cell; sFRP1 = secreted Frizzled-related protein 1; Stat = signal transducer and activator of transcription; TCF = T-cell factor; TF = transcription factor.

impact on Myc levels, it is likely that Myc has a variety of functions throughout normal mammary gland development, downstream of one or more of these inputs. To date, however, there are only a few studies on the physiological role of Myc in the mammary gland. In this review we present what is known about Myc from transgenics and conditional knockout models, and also include indirect evidence implicating Myc based on results from other studies. We shall discuss the potential inputs activating Myc during development and the ensuing outputs of Myc activity. A summary of the discussion on the role of Myc in normal development is shown in Figure 1.

Embryogenesis

Development of the mammary gland begins at embryonic day 10 as an appendix of the ventral skin, with formation of the milk line followed by appearance of placodes [10]. The ErbB4 ligand, Neuregulin3 (Nrg3), was identified as a specification signal for placode formation. Based on this, Keratin14 (K14)-Nrg3 transgenic mice expressing Nrg3 throughout the basal layer of the epidermis, including the stem and progenitor cells, were investigated [11]. Ectopic Nrg3 expression resulted in hyperplastic epidermis and formation of supernumerary placodes. Interestingly, the skin of K14-Nrg3 mice displayed increased Myc expression and decreased levels of α_6-integrin and β_1-integrin, which are adhesion receptors highly expressed in adult mammary stem cells (SCs) [12]. While the direct stimulus of Myc expression is unknown, it seems to be an important mediator of the phenotype observed in K14-Nrg3 mice, as strong similarities were found in a K14-Myc model where Myc is activated in the epidermis [11]. Nrg3 therefore possibly has a role in promoting mammary lineage commitment and in the regulation of SC fate via Myc.

Mammary stem cells

While these results suggest a role for Myc in embryonic development, its role in adult mammary SCs has not yet been analyzed. A function for Myc in mammary SCs seems likely, however, based on its role in other well-characterized models [1,13]. In the hematopoietic system, the balance between SC self-renewal and differentiation is controlled by Myc levels, which in turn regulate expression of adhesion molecules such as N-cadherin and β_1-integrin [14]. In addition, the Wnt and Notch signaling pathways have been proposed to play important roles in mammary SCs [13], and their effector proteins – β-catenin/TCF and Notch intracellular domain (N^{IC})/Cbf1, respectively – each have binding sites on the Myc promoter [3]. Furthermore, a transgenic model expressing stabilized β-catenin in basal cells, which are believed to contain the SC population [12], displayed upregulation of Myc [15]. Finally, the HC11 mammary epithelial cell line, which has SC-like properties, might be an interesting model to explore Myc function, since Myc levels are downregulated when these cells are induced to differentiate [16].

Puberty and pregnancy

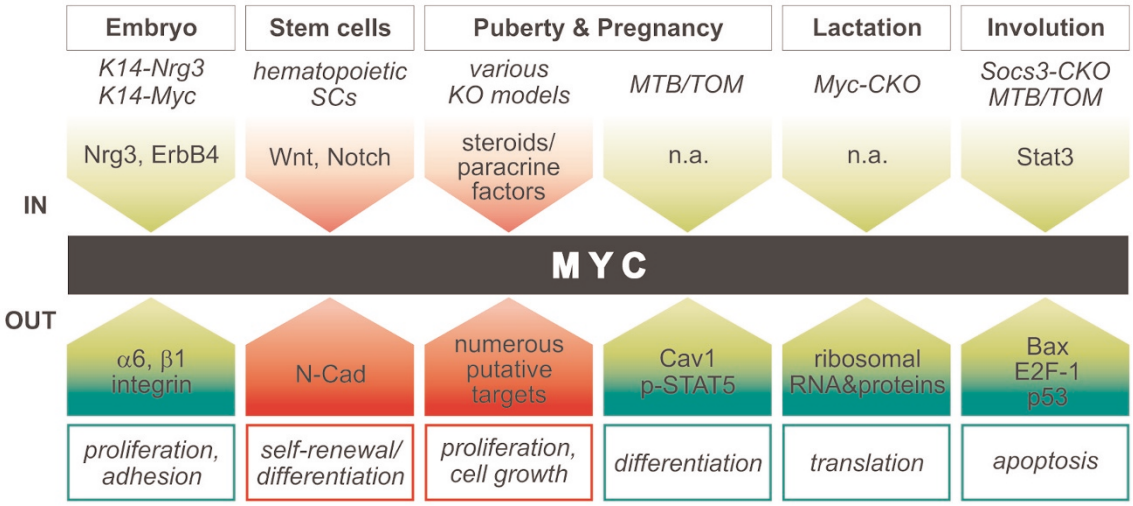
The steroid hormones estrogen and progesterone, as well as the prolactin receptor binding peptides prolactin and placental lactogen, dominate the extensive developmental changes occurring during puberty and pregnancy [4,17]. Both estrogen and progesterone are able to directly stimulate Myc expression via an estrogen response element [18] and a progesterone receptor regulatory element [19]. While Myc is expressed at low levels in prepubertal and virgin glands, it reaches its highest expression levels between days 6.5 and 12.5 of pregnancy, after which the RNA level slowly returns to baseline until parturition [20].

Interestingly, Myc was shown to be directly downstream of estrogen and progesterone in breast cancer cells, stimulating their proliferation [21,22], but one of the major differences between normal breast and malignant breast is that the estrogen- and progesterone receptor-positive cells do not proliferate during normal development. Instead, by producing paracrine mediators, estrogen and progesterone stimulate proliferation of neighboring cells via amphiregulin [23] and Wnt4 [24], respectively. Other growth factors, such as epidermal growth factor or receptor activator of NF-κB ligand, have also been shown to act as paracrine mediators during mammary gland development [25]; and importantly, Wnt, epidermal growth factor and receptor activator of NF-κB ligand might all affect Myc levels directly or indirectly [3].

Although no study has yet addressed the contribution of Myc to pubertal development and early pregnancy, it is very likely that Myc is induced via paracrine signals, and, at least early in pregnancy when Myc levels are highest, Myc might promote proliferation. Importantly, different Myc targets such as cyclin-dependent kinase (Cdk) 4, nucleophosmin and nucleolin are also highly expressed at this time [20] – prompting us to speculate that Myc might not only have a direct role in proliferation, but also in the synthesis of ribosomal components required for rapid growth during pregnancy.

A transgenic model revealed why it is important for Myc levels to decrease, starting at 12.5 days of pregnancy, and to remain low until parturition. Using a doxycycline inducible model (MMTV-rtTA/TetO-MYC (MTB/TOM)) [26], it was shown that transient overexpression of Myc between days 12.5 and 15.5 of pregnancy induced a lactation failure [27]. Abnormal Myc expression was shown not only to induce proliferation, but also to promote precocious Stat5 activation and differentiation, followed by premature involution, triggered by milk stasis. Decreased levels of Caveolin1 (Cav1), a direct target of Myc repression [7], were shown to be responsible for the phenotype. Cav1 is a negative regulator of Janus kinase2–Stat5 signaling, and $Cav1^{-/-}$ mammary epithelial cells show hyperactivation of Stat5 and spontaneous milk production [28], similar to what is observed when Myc is elevated late in pregnancy.

Figure 1



Ins and outs of the 'black box' MYC during normal mammary gland development. The diagram displays the models (italic, top) used to investigate the various inputs and outputs of Myc (green boxes). Speculations based on other model systems that have not yet been shown in the mammary gland are presented in red. Inputs are signaling molecules that are known or suggested to impact on Myc levels; inputs are not applicable (n.a.) in transgenic models with genetically deregulated Myc levels. The outputs are, where available, direct targets of Myc transcriptional activity and general biological functions described for Myc at the specific developmental stage (italic, bottom). During embryogenesis, transgenic expression of Neuregulin3 (Nrg3), a major factor controlling mammary placode development, induced high Myc levels, thereby changing the proliferative and adhesive properties of cells [11]. The speculated role of Myc in mammary stem cells (SCs) is mostly based on data from hematopoietic SCs and the known importance of Wnt and Notch pathways in other SC types [13]. Myc's role during puberty and early pregnancy has not yet been analyzed, but as various steroids and paracrine factors can induce its expression [3] Myc might play a role in promoting proliferation and cell growth via its numerous cell cycle and translation-related targets. A transgenic mouse model (MMTV-rtTA/TetO-MYC (MTB/TOM)) revealed that Myc overexpression during late pregnancy leads to precocious proliferation and differentiation via repression of Caveolin1 (Cav1) and signal transducer and activator of transcription (Stat) 5 hyperactivation [27]. Despite its low levels during lactation, Myc has an important role in mRNA translation, as shown in our own laboratory using mammary glands conditionally lacking Myc (Myc-CKO) [29]. Finally, in Socs3 conditional knockout (CKO) mice it was shown that increased Stat3 activation leads to accelerated apoptosis via high levels of Myc, suggesting a direct role for Myc downstream of Stat3 in involution [31]. More detailed discussion can be found in the text. K14, Keratin14; KO, knockout; N-Cad, N-cadherin.

These results demonstrate an important characteristic of Myc – namely, the effects of Myc are dependent upon the developmental stage of the mammary gland. Myc overexpression between 12.5 and 15.5 days of pregnancy was necessary and sufficient to induce the observed phenotype, while overexpression during other short intervals (for example, 9.5 to 12.5 days) did not result in lactation failure [27]. Deregulated Myc therefore leads to a premature decrease in Cav1, thereby removing its restraining influence on prolactin receptor–Janus kinase2–Stat5 signaling.

Lactation

In many cell types Myc is downregulated when cells undergo terminal differentiation. Indeed, Myc RNA levels do drop during lactation to below those levels found in the virgin gland [20]; however, the molecular reason for this dramatic decrease is not known. The mammary gland as a milk factory produces immense amounts of lipids, lactose and proteins, and most of its energy is devoted to milk component synthesis. Considering the importance of Myc in energy and glucose metabolism as well as ribosome biogenesis and translation [1], it is possible that, even despite its low levels, Myc has an essential function during lactation.

Indeed, data from our laboratory revealed a novel role for Myc in the mammary gland using a conditional knockout approach. In *c-myc*$^{fl/fl}$*WAP(whey acidic protein)iCre* mice, loss of Myc occurs exclusively in luminal alveolar cells starting from mid-pregnancy. We show that milk production was reduced in Myc mutant mothers, while milk composition was unchanged between wild-type and mutant mothers [29]. Electron microscopy revealed that there were less secretory vesicles budding from the endoplasmic reticulum in lactating mutant cells, suggesting a decreased protein synthesis. In polysomal fractionation experiments we found that translation efficiency was generally decreased in lactating, Myc-deficient mammary glands. Furthermore, we observed reduced expression levels of ribosomal proteins and RNA, as well as proteins involved in translation and ribosome biogenesis. Although compensation by N-Myc or L-Myc cannot be

excluded, neither was found upregulated in c-Myc mutant glands. These results highlight Myc's importance for mammary gland function even when endogenous levels are low.

Involution

The impact of Myc on apoptosis has been widely studied in many systems [2]. In the mammary gland, the high levels of apoptosis during the first phase of involution are promoted by the leukemia inhibitory factor–Stat3 axis [30]. Compared with its low expression in lactating glands, higher Myc levels are detected during involution [20]. Importantly, a role for Myc during the first apoptotic phase was uncovered in mice with a conditional deletion of Socs3 (*Socs3*$^{-/fl}$*WAPiCre*), a negative regulator of leukemia inhibitory factor–Stat3 signaling [31]. Socs3-deficient glands displayed accelerated apoptosis accompanied by elevated levels of p-Stat3 and Myc, which is a direct Stat3 target gene.

To further analyze Myc's function in apoptosis, the doxycycline-inducible MTB/TOM model described above [26] was used, giving more evidence for a direct role of Myc in involution. Overexpression of Myc prior to forced weaning caused a dramatic acceleration of involution, accompanied by increased apoptosis and high levels of the pro-apoptotic proteins Bax, E2F-1 and p53, which have all been described as direct or indirect Myc targets.

The conclusions based on those two models suggest that Myc acts as a central mediator of apoptotic signaling in the mammary gland, being a direct target of Stat3 and inducing expression of pro-apoptotic genes.

Background on Myc in breast cancer

Alterations in Myc have been found in many types of tumors. At the genomic level these include gene amplification, chromosomal translocations and point mutations. Furthermore, Myc is regulated by multiple signals that control promoter activity, transcriptional elongation and translation, as well as by post-translational modifications that control Myc's transcriptional targets as well as protein stability. Since most tumors have numerous alterations in signaling cascades, Myc is likely to be deregulated by some mechanisms in most cancers.

Considering breast cancer, amplification is the most commonly described alteration. The *MYC* amplicon on chromosome 8q23-24 was one of the first consistent genetic alterations found [32]. Results from a meta-analysis of breast tumors yielded a frequency of 15.7% for the *MYC* amplicon, with a range of 4 to 52% depending on the study [21,33]. *MYC* amplification is found in a high proportion of tumors with Brca1 alterations, as well as in ERα-negative, basal-like tumors [34,35]. Despite intense screening efforts, point mutations of Myc have not been described in breast or other carcinomas [36]. Other mechanisms promoting increased Myc levels, however, have been found. The ubiquitin ligase F-box and WB repeat domain containing 7, which catalyzes polyubiquitination of Myc and ensuing degradation, is often mutated or downregulated in breast cancer [37]. Moreover, the de-ubiquitinating enzyme ubiquitin-specific protease 28 – which antagonizes F-box and WB repeat domain containing 7, thereby stabilizing Myc – has been found overexpressed in a small panel of breast tumors [38]. In addition to these breast tumor-specific alterations, it is possible that Myc is deregulated in most breast tumors since the normal tight control that is exerted on Myc at multiple levels is impaired in essentially all cancer cells.

What is the output of deregulated Myc in breast cancer? Myc levels respond to proliferative and anti-proliferative stimuli, and many reported Myc target genes, such as cyclin D_2, Cdk4 and the Cdk inhibitor $p21^{Cip1}$, are important regulators of proliferation [2]. A major mechanism underlying Myc's role in breast cancer is activation of cyclin E–Cdk2 via repression of $p21^{Cip1}$ [21]. Myc deregulation not only impacts on proliferation, but on various other processes like survival and apoptosis.

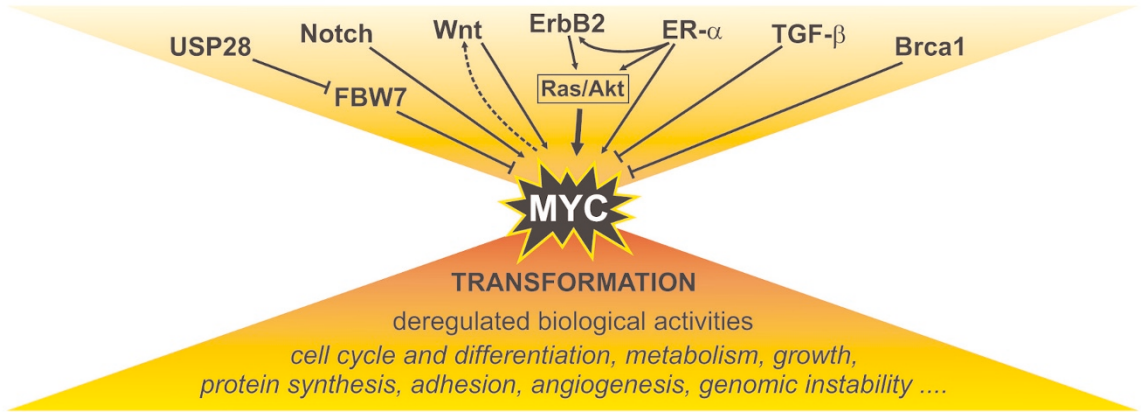
Here we would like to mention Myc and translational control, since we have recently shown an important role for Myc in translation also during mammary gland development [29]. Generation of the Eμ-Myc B-cell leukemia model in mice with heterozygosity in the gene encoding the L24 ribosomal protein restored normal levels of protein synthesis in the leukemic cells, thereby suppressing Myc's oncogenic potential [39]. These results show that, in addition to proliferative effects, abnormal Myc activation also deregulates protein synthesis, which in this model is necessary for oncogenesis.

Considering the broad-ranging effects of Myc, the outcome of its activation in breast cancer is likely to be dependent upon the cellular context. Indeed, using a siRNA approach to knockdown Myc in a panel of breast cancer cell lines, and combining this with a genomic and phenotypic analysis, revealed that selectively regulated target genes in each cell line were responsible for the differential effects resulting from Myc loss. A comprehensive list of potential Myc targets in the BT-474, MCF-7 and MDA-MB-231 breast cancer cell lines can be found in Cappellen and colleagues' study [40]. A summary of the discussion on the role of Myc in cancer is shown in Figure 2.

Myc and mammary cancer

Myc was the first oncogene tested for mammary tumor-forming potential using the mouse mammary tumor virus (MMTV)-long terminal repeat to drive its expression. Tumor incidence in MMTV-Myc transgenic females was high; however, the kinetics of tumor appearance suggested that Myc expression alone was not sufficient to induce cancer [41]. Indeed, double transgenics expressing Myc and mutant Hras showed more rapid mammary tumor development [2]. More recently, it was shown that Myc induction using the

Figure 2



Aberrant Myc expression causes mammary cancer. Myc is deregulated in most mammary tumors by multiple mechanisms, including gene amplification, or aberrant expression due to alterations in signaling pathways that influence Myc RNA or protein levels as well as its transcriptional activity. Each of the indicated proteins or pathways impacts on Myc expression or activity in mammary cancer. Specifically, the Notch and Wnt pathway effectors, Notch intracellular domain/C promoter-binding factor 1 and β-catenin/T-cell factor, respectively, as well as estrogen receptor alpha (ERα), bind the Myc promoter, thereby stimulating transcription. Dysregulation of transforming growth factor beta (TGFβ) and Brca1 in mammary cancer has been reviewed recently [34]. TGFβ, via Smads, suppresses Myc expression, while Brca1, which is frequently deregulated in ERα-negative, basal-like breast cancer, normally blocks Myc transcriptional activity. The ubiquitin-specific protease ubiquitin-specific protease 28 (USP28) was found overexpressed in breast tumors [38] and stabilizes Myc via antagonizing F-box and WB repeat domain containing 7 (FBW7), which is frequently lost or mutated in breast tumors [37]. Finally, ErbB2 activation, which is also regulated by ERα, stimulates pathways like rat sarcoma/extracellular signal-related kinase (Ras/Erk) and phosphoinositide 3-kinase/serine/threonine kinase Akt (PI3K/Akt) that influence Myc RNA and protein levels. See text for further details. Myc is an activator of RNA polymerase II-driven transcription for multiple target genes [2] and also affects RNA polymerase I- and III-mediated transcription, thus regulating ribosome biogenesis and translation. In cancer cells, the outcome of deregulated Myc will be wide-ranging considering that Myc influences the cell cycle, protein synthesis, cell growth and metabolism, cell death, genomic instability, tumor-induced angiogenesis, adhesion, as well as other cellular functions. This is exemplified by examining the effects of Myc knockdown in breast cancer cell lines, where a genomic and phenotypic analysis revealed that selectively regulated target genes in each cell line were responsible for the differential effects resulting from Myc loss [40].

inducible MTB/TOM model described above [26] results in mammary tumors, with approximately one-half also harboring activating Kras2 mutations. Interestingly, these tumors did not regress following Myc deinduction, demonstrating that Hras mutations not only change tumor kinetics but also cause progression to Myc independency [26].

Myc and Notch

Each membrane spanning Notch receptor is proteolytically processed in response to ligand binding, releasing N^{IC}, which converts the nuclear Cbf1 repressor to a transcriptional activator. A link between aberrant Notch signaling and mammary cancer was first discovered in MMTV-induced tumors with proviral DNA integrated within the Notch4 gene, leading to constitutive N^{IC} expression. Different mechanisms activate Notch signaling in human breast cancer [42,43]. For example, coexpression of Jagged1 ligand and Notch receptors has been found in breast cancers, in particular the triple-negative (negative for ERα, for progesterone receptor and for ErbB2) subtype [44], suggesting an autocrine mechanism of Notch pathway activation. Furthermore, levels of Numb, a negative regulator of Notch, were shown to be low in ~50% of primary breast tumors [45], which could contribute to maintenance of pathway activity.

Based on the observation that mammary tumors in the MMTV-N^{IC} transgenics presented elevated Myc, its role in Notch transformation was examined in mice with floxed Myc alleles [43]. Conditional ablation of Myc using the WAPCre transgene revealed that Myc was indispensable for development of N^{IC}-driven mammary tumors. This contribution of Myc to Notch-induced tumorigenesis is interesting especially when comparing it with Wnt pathway-driven models (see below). Moreover, Myc was shown to be a direct target of the Notch pathway, since a complex of N^{IC} and Cbf1 was detected on the Myc promoter. The Cbf1 binding site on the human Myc promoter is conserved, and immunohistochemistry revealed that there was a significant correlation between high Myc levels and N^{IC} in human breast tumors [43]. It is intriguing that coexpression of Jagged ligand and Notch receptors is found in triple-negative breast tumors [44], a subgroup that also has high Myc activity [46].

Myc and the Wnt pathway

Wnt1 was the first identified oncogene activated by MMTV insertional mutagenesis. Wnt-mediated activation of the canonical pathway leads to β-catenin stabilization, TCF binding and transcriptional activation of Myc. Mammary tumors arising in Wnt1 transgenics [47] and models driven

by a β-catenin-stabilized mutant [15] show elevated levels of Myc. Human breast tumors, unlike colon cancer, do not possess Wnt pathway-activating mutations. Deregulation of Wnt signaling appears to occur by autocrine mechanisms, however, since multiple Wnt ligands and Frizzled receptors are coexpressed [48], and the negative Wnt pathway regulator – secreted Frizzled-related protein (sFRP1) – is often absent [49]. A positive feedback loop has also been described for Myc and the Wnt pathway. In telomerase-immortalized, Myc-transformed human mammary epithelial cells, Myc was shown to repress sFRP1 and Dickkopf 1, another negative pathway regulator, thereby contributing to activation of canonical Wnt signaling [50]. Along the same lines, Myc knockdown in MDA-MB-231 tumor cells increased Dickkopf 3 expression [40].

What is Myc's role in tumors induced by Wnt pathway activation? The dependency of Wnt-driven mouse mammary tumors on Myc expression has not been tested. In other tumor models driven by adenomatosis polyposis coli (APC) loss, the importance of Myc has been examined using organ-specific Cre recombinase-mediated deletion of floxed Myc alleles. In the intestines, Myc deletion reversed the tumor phenotype induced by APC loss, and it was shown that the majority of Wnt targets in the intestines were Myc dependent [51]. In striking contrast, Myc deletion had no affect on the phenotype of APC loss in the liver, where most Wnt target genes were β-catenin dependent but Myc independent [52].

These two studies [51,52] reveal that the importance of Myc in a particular tumor model is very specific and can differ as already discussed for the normal organ-specific Myc functions. As discussed above, deregulated Myc might enforce autocrine Wnt pathway activity in human tumors by repressing negative regulators such as sFRP1. Other potential roles for Myc have not been examined; however, blockade of the Wnt pathway usually results in lowering Myc levels. Stable expression of sFRP1 in MDA-MB-157 and MDA-MB-231 breast tumor cell lines blocks proliferation of both cell lines, and Myc RNA was decreased in the former [53] while Myc protein was lower in the latter [49]. In a panel of breast cancer cell lines, siRNA-mediated knockdown of Dishevelled, an essential mediator of Wnt signaling, led to lower Myc and decreased proliferation in most cell lines [54].

In summary, the current data suggest that, in mouse and human mammary tumors with constitutive Wnt signaling, Myc levels are elevated and might have a role in transformation.

Myc and ErbB2

Amplification of *ERBB2* leading to receptor overexpression is found in 20 to 25% of primary breast tumors. In these tumors, constitutive ErbB2 activation stimulates numerous intracellular signaling pathways including Ras/Erk and PI3K/Akt, both of which impact on Myc transcription and protein stability. The role of Myc has been examined in the ErbB2-overexpressing SKBr3 and BT-474 breast tumor cell lines. Treatment of both with the ErbB2-specific antibody trastuzumab caused a cell cycle block that was accompanied by a decrease in PI3K/Akt pathway activity, and by downregulation of Myc and D-type cyclins [55]. Interestingly, ectopic expression of Myc in SKBr3 cells partially rescued the cells from functional ErbB2 inactivation [56], pointing to the importance of Myc as an ErbB2 effector.

Myc and estrogen receptor alpha

In the normal breast, both rodent and human, a major role of the ERα-positive cells is to act as sensors to relay a proliferative signal to neighboring cells. In contrast, many breast tumor cells are ERα-positive, and they have not only acquired the potential to proliferate in response to steroid hormones but are also dependent upon them for survival [21,57]. Since Myc is an ERα target, it is important to understand whether Myc has a role in acquisition of this phenotype. Moreover, since patients whose tumors are ERα-positive are treated with anti-estrogen therapy, Myc's role in response or resistance is also of high interest. Unfortunately, consistent clinical data relating *MYC* amplification or expression levels to endocrine therapy response are not available [21]. Overexpression of ErbB2 has been correlated with *de novo* and acquired endocrine resistance [57], however, and Myc is an ErbB2 effector.

The role of Myc in ERα signaling has been well characterized in the MCF-7 breast tumor cell line [21]. Myc RNA increases rapidly in response to estrogen treatment of these cells, and Myc knockdown impairs the ability of estrogens to stimulate proliferation. Furthermore, Myc overexpression in cells arrested by ERα antagonists overcomes the proliferative block. Interestingly, adaptation of MCF-7 cells to growth in estrogen-deprived medium is associated with upregulation of ERα-regulated target genes, including Myc [58], suggesting a mechanism whereby Myc maintains an important role in proliferation and survival even in the absence of ERα activity. It should also be mentioned that ERα cross-regulates ErbB2, which in turn impacts on Myc via activation of downstream signaling pathways [57].

From transcriptome and network analyses, the close link between Myc and ERα signaling has become even more apparent. MCF-7 cells show a high level of overlap between ERα-regulated and Myc-regulated genes. Indeed, more than 50% of the estrogen-responsive genes are also Myc targets [59]. Moreover, a meta-analysis of transcriptional and pathway data, carried out on primary breast tumors, revealed that Myc activity is elevated in ERα-negative, basal-like breast cancers, as measured by target gene levels [46].

In summary, these data led to the proposal that elevated Myc activity present in ERα-negative breast tumor cells mimics the activity of estrogen on ERα-positive breast tumor cells [46,59].

Myc as a prognostic, predictive or therapeutic target in breast cancer

Considering that Myc deregulation is so common in breast cancer, Myc has been examined as a prognostic factor and as a predictive factor. Indeed, *MYC* amplification is associated with aggressive clinical features including high grade and lymph node positivity, and correlates with poor patient outcome [34]. A major interest in breast cancer is the use of genetic alterations to categorize patients into treatment groups; a good example being detection of the *ERBB2* amplicon for trastuzumab treatment. Gene expression signatures are also being generated in order to provide predictive data on patient response to standard chemotherapeutics and to targeted therapy. In one study of chemotherapy-treated breast cancer patients, an attempt to correlate Myc pathway activity with response yielded inconsistent results. The group with Myc and Ras pathway activation had a high percentage of responders, while patients whose tumors had Myc and E2F pathway activity responded poorly [60]. These results underscore the difficulty of using Myc levels alone as a predictive or prognostic factor, and emphasize the fact that the cellular context of Myc expression and activity determine the outcome.

Approaches to target Myc activity in tumors are also under consideration [34,61]. Although targeting Myc is appealing, there are many difficulties associated with altering transcription factor activity. Considering the variety of kinase inhibitors currently available, it is worth considering their use in breast tumors with Myc deregulation. Based on the molecular concepts analysis [62] of the Myc pathway activation signature in cancer, and on the identification of the genes downregulated in MCF-7 cells treated with wortmannin and LY-294002, there is reason to believe that PI3K inhibitors might be especially potent in breast cancers with high Myc activity. As discussed [46], ERα-negative, basal-like breast tumors with high Myc activity might be particularly susceptible to PI3K inhibitors.

Conclusion

Clearly, there are numerous signaling events in the development of the mammary gland that can be mediated, at least in part, via Myc. Using transgenic models, functions for Myc – some potential, others data based – have been discussed for embryonic development, pregnancy, lactation and involution. Furthermore, results from other model systems suggest that Myc could play a role in stem cell fate, as well as during early pregnancy, where Myc expression levels are highest in the normal gland. Even more efforts have been made to investigate Myc's role in transformation, as deregulation of Myc via amplification, overexpression or stabilization of the protein is a frequent event in breast cancer. Signaling pathways implicated in breast cancer, such as ERα, ErbB2, Notch and Wnt, all contribute to aberrant Myc levels or activity. The challenge for future studies will be to reveal the suitability of targeting Myc for breast cancer treatment, either by direct inhibition or by indirectly targeting another pathway.

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Competing interests

The authors declare that they have no competing interests.

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