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Myc and Mammary Cancer: Myc is a Downstream Effector of the ErbB2 Receptor Tyrosine Kinase

The proto-oncogene *c-myc* encodes a transcription factor which plays a major role in the regulation of normal cellular proliferation and is aberrantly expressed in many breast tumors. In a normal cell Myc expression levels are tightly regulated being subject to many layers of control. Errantly expressed Myc collaborates with other oncogenes to promote transformation. In this review we will focus on the association between abnormal Myc expression and mammary cancer. In particular, we will discuss the role of Myc as a downstream effector of the ErbB2 receptor tyrosine kinase which is overexpressed and constitutively activate in many mammary tumors. The cooperation between Myc and ErbB2 in transformation will be discussed in relation to clinical studies on Myc in human cancer and with consideration of transgenic models of Myc-induced mammary cancer. Data from our laboratory will be presented showing that deregulated ErbB2 activity strongly stimulates cytoplasmic signaling pathways which in turn impinge on Myc at multiple levels causing its deregulated expression.

KEY WORDS: EGF receptor family; transgenic mice; $p27^{Kip1}$; apoptosis; transcription factor.

INTRODUCTION

Cancer arises as a result of cumulative alterations in the genetic make-up of somatic cells. Such alterations principally lead to the aberrant expression, mutation or deletion of proteins involved in the modulation of intracellular pathways governing normal cellular proliferation and differentiation. These defects allow cancer cells to evade signals eminating from their external environment or from internal checkpoint controls through deregulation of signaling pathways which normally keep cell proliferation and survival tightly controlled. In this context, the proto-oncogene *c-myc*, which encodes a transcription factor playing a major role in the regulation of normal cell proliferation, is abberantly expressed in many human cancers. Indeed, taking breast cancer as an example, *c-myc* amplification was one of the first consistent genetic alterations discovered in primary human tumors (1).

Myc is a member of the bHLHZip[4] family of transcription factors which, when dimerized with its partner Max, binds to specific DNA sequences resulting in the transcriptional regulation of target genes involved in the control of cellular growth/proliferation [reviewed (2–8)]. All known biological effects of Myc, including the ability to transform cells, result from its activity as a transcription factor. It has been proposed that Myc acts as a sensor of the cellular environment (9), able to trigger proliferation and, in stress situations, apoptosis. Furthermore, when deregulated, Myc

[1] Friedrich Miescher Institute, PO Box 2543, CH-4002 Basel, Switzerland.
[2] Novartis Pharma, K-125.1317, CH-4002 Basel, Switzerland.
[3] To whom correspondence should be addressed. e-mail: hynes@fmi.ch

[4] Abbreviations: epidermal growth factor (EGF); receptor tyrosine kinase (RTK); phosphatidylinositol-3′ kinase (PI3K); endoplasmic reticulum (ER); mitogen-activated protein kinase (MAP kinase); basic helix-loop-helix zipper (bHLHZip); Myc Box II (MbII); transactivation/transformation-domain associated protein (TRRAP); histone acetyltransferase (HAT); histone deacetylase (HDAC); human mammary epithelial cells (HMEC); human telomerase catalytic subunit (hTERT); cyclin-dependent kinase inhibitor (CKI); monoclonal antibody (mAb); mouse mammary tumor virus (MMTV); whey acidic protein (WAP); transforming growth factor α (TGFα).

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has characteristics which would clearly contribute to its transforming ability; including its ability to drive cells through the G1/S-transition of the cell cycle as well as connections between Myc and the induction of genomic instability.

In this short review we have focused on the connection between aberrant Myc expression and mammary tumorigenesis. Emphasis is placed on the biological activities of Myc, clinical studies addressing Myc deregulation in human breast cancer and the application of transgenic models of Myc-induced mammary cancer. Additionally, based on our own experimentation, the role of Myc as a downstream effector of the ErbB2 receptor tyrosine kinase (RTK) will be discussed (10,11). ErbB2 is activated due to gene amplification and overexpression in a high percentage of human breast tumors. Through antibody-mediated downregulation of ErbB2 receptor signaling pathways in ErbB2-overexpressing breast tumor cell lines, we suggest that a major downstream effector contributing to the potentiation of ErbB2-dependent tumor cell proliferation is Myc. This model will be discussed in relation to clinical aspects of the impact of Myc and ErbB2 deregulation in mammary carcinogenesis, and the application of ErbB2-directed tumor-specific therapies.

BIOLOGICAL ACTIVITIES OF Myc

Myc, A Key Regulator of Proliferation and Apoptosis

Myc has a central role in normal cellular growth/proliferation control. This is most dramatically demonstrated by the observation that expression of exogenous Myc in quiescent fibroblasts induces S-phase entry in the absence of growth factors (12). Furthermore, Myc-negative fibroblasts proliferate at a slower rate and exhibit reduced expression of G_1 cell cycle regulators (9). Myc has been suggested to act as an environmental sensor, coordinating adjustment of cell cycle progression to the external milieu by affecting cell cycle regulators at multiple levels (9). Consistent with a role in proliferation *c-myc* transcription is low in resting or differentiated cells, is rapidly induced following mitogenic stimulation and is required for continuous cellular proliferation (4,5). In the case of the mammary gland, similar fluctuations in *c-myc* RNA levels are observed; reflecting the proliferation index of the gland. Indeed, the *c-myc* promoter is a target for peptide growth factors and for estrogen (4,13), both of which contribute to proliferation of the gland. Myc mRNA levels increase during pregnancy-related proliferation (14). In addition to elevated expression during pregnancy, increases in Myc expression may also be important during the proliferative changes which occur in the breast during the menstrual cycle (15). In contrast, Myc is not detected during lactation (14), reflecting the differentiated state of the gland at this time.

A second distinctive characteristic of Myc is its ability to induce apoptosis. This was discovered following growth factor-withdrawal from cells ectopically expressing Myc. In this case, DNA synthesis occurred, but cells failed to multiply and apoptosis was accelerated (16). It is now known that deregulated Myc expression sensitizes cells to diverse proapoptotic stimuli [reviewed (17)]. Indeed, it has been suggested that a cell is constantly poised to enter apoptosis and requires stimuli, either intracellular or from the external environment, in order to survive and proliferate (17). In order for tumor cells to proliferate in the presence of deregulated Myc expression, therefore, additional genetic alterations must occur, which would allow the malignant cells to circumvent proapoptotic stimuli. It should also be mentioned here that Myc probably plays a role in regulated apoptosis occurring under normal physiological conditions. In this context, during murine mammary gland involution following pup withdrawal, *c-myc* RNA levels have been observed to increase (13). It is tempting to speculate, therefore, that this upregulation is linked to the massive apoptosis which occurs during this developmental stage.

Myc the Transcription Factor

Myc is a member of the bHLHZip family of transcription factors, possessing a C-terminal DNA binding domain and a N-terminal transactivation domain. Myc dimerization with its partner Max is essential for DNA binding to a consensus site, the so-called E-box, and transcriptional regulation of target genes. The N-terminus of Myc contains two highly conserved regions, the Myc box (Mb) I and II domains. All biological properties of Myc, including those associated with transformation, require its DNA binding domain and dependent upon the cellular context, the N-terminal MbI and/or MbII domains, making it probable that Myc exercises its biological effects

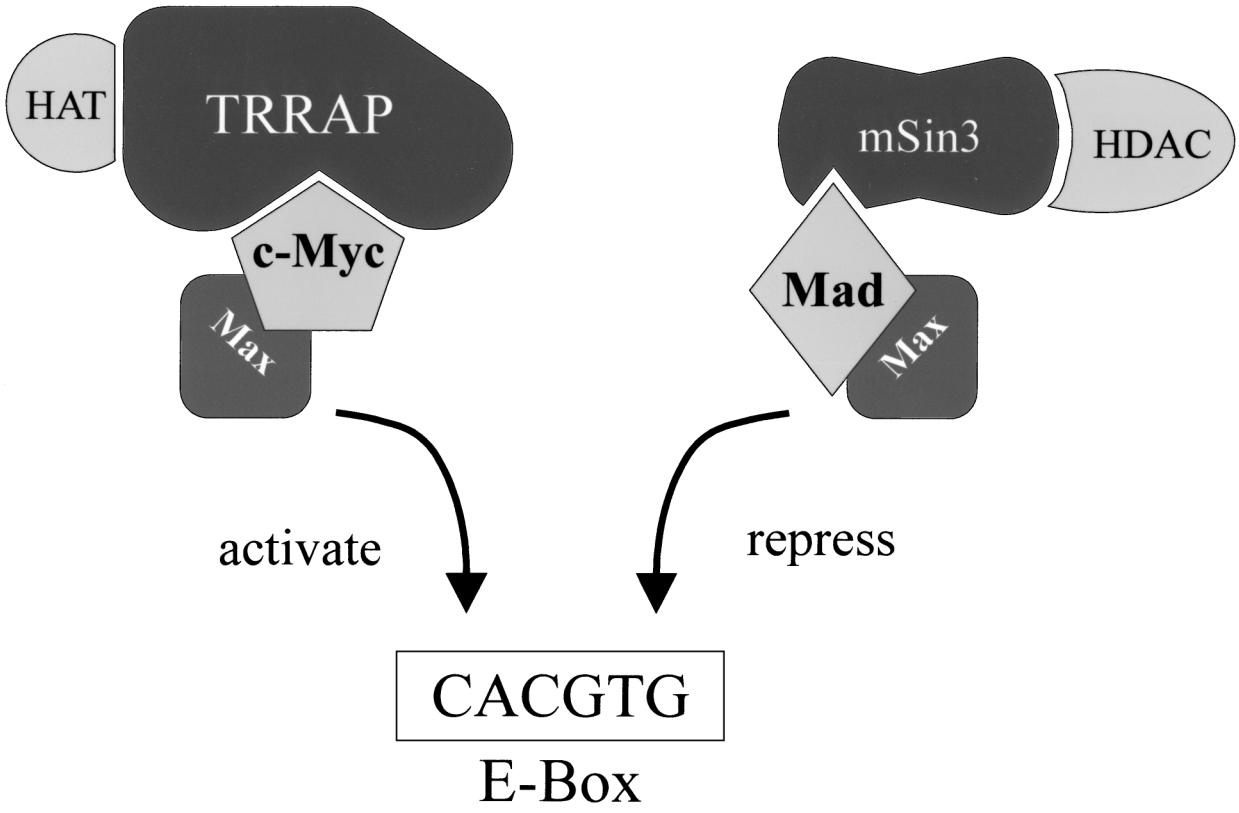

Fig. 1. A model of the contrasting functions of Myc/Max and Mad/Max dimers. Myc/Max dimers activate transcription via recruitment of TRRAP and a HAT to the consensus E-box found in target genes. Mad/Max dimers oppose Myc function via recruitment of a multisubunit complex containing HDAC to the same E-box (see text and Ref. 19 for further details).

through modulation of target gene expression. The MbII domain binds the nuclear cofactor TRRAP (18), which in turn recruits the HAT hGCN5 (19). Recruitment of this chromatin remodeling complex to the DNA by Myc provides an explanation for Myc/Max induced transcriptional activation. Myc/Max activity is opposed by Max, when dimerized with one of the Mad family members. Mad proteins bind a complex containing co-repressors and HDACs (2,3). Since Mad/Max dimers and Myc/Max dimers recognize the same DNA sequence, a very simplistic model proposes that Mad-Max dimers, via recruitment of HDACs, antagonize the transcriptional activity of HAT-associated Myc-Max complexes (19) (Fig. 1). A final point to consider is that the balance of the respective dimers may determine Myc target gene expression, particularly as Myc is expressed in proliferating cells and is down-regulated in differentiated cells, while the opposite tends to be true for the Mad family members (5). Myc and Mad1 protein expression have been examined in normal human breast and in progressive breast disease, where this correlation was observed. Mad1 expression was high in differentiated cells and decreased in high-grade tumors, while the inverse was observed for Myc levels (20).

A comprehensive understanding of the mechanism underlying Myc's biological activities will arise from the identification of Myc target genes. These have remained rather elusive, despite intense study, for various reasons. However, the general consensus is that the identified targets reflect Myc's functions and can be catagorized as targets involved in growth control and apoptosis, including among others: hEST2, the catalytic subunit of telomerase; $p19^{Arf}$, the tumor suppressor; thymidine kinase, involved with DNA metabolism; and lactate dehydrogenase-A, a participant in anaerobic glycolysis. For a detailed discussion of Myc targets and their identification, the reader is referred to recent reviews (2,3). Meaningful for the discussion of our results (see later), is the fact that important cell cycle regulators, such as the D-type cyclins, are transcriptionally regulated by Myc (21,22).

Myc AND MAMMARY CANCER

Mechanisms Leading to Deregulated Myc Expression in Cancer

In a normal cell *c-myc* transcription is tightly controlled and dependent upon proliferative stimuli. Furthermore, *c-myc* mRNA and Myc protein are both short-lived [reviewed (4)]. Thus, in a normal cell Myc expression is highly regulated, at all possible levels. In sharp contrast to this, deregulated Myc expression is quite prevalent in human tumors. Deregulation arises through diverse mechanisms, including *c-myc* amplification in solid tumors, and translocations in leukemias (23). One of the first genetic alterations reported in human breast tumors was *c-myc* amplification (1). Since then, *c-myc* amplification has been extensively studied and the current consensus is that it occurs in approximately 20% of all breast cancer cases [reviewed (24)]. However, examination of *c-myc* mRNA in a relatively small number of breast tumors by RT-PCR has suggested that *c-myc* overexpression does not necessarily arise from gene amplification (25). Although the mechanism was not further examined, the results are not suprising considering that Myc expression is controlled by transcriptional as well as post-transcriptional mechanisms.

Myc protein levels are also tightly regulated. Mutations within *c-myc* occur in a high percentage of Burkitt's and other lymphomas (26). It has recently been shown that some of these mutations lead to stabilization of the Myc protein, preventing its destruction by ubiquitin-mediated proteolysis (27). Stabilizing Myc mutations have not been reported in breast cancers. However, Myc protein levels are also controlled by cytoplasmic signaling pathways which are often deregulated in breast tumor cells. In this context, two pathways which are highly activated due to overexpression of the RTK ErbB2 in breast tumors are the PI3K and MAP kinase pathways. Intriguingly, the PI3K pathway has been implicated in the translational induction of Myc (28), and high MAP kinase activity promotes an increase in the stability of Myc protein (29). Considering that the ultimate level of Myc protein is subject to so many layers of control and that tumor cells have alterations in many signaling pathways which impact on this, it would not be suprising to find that Myc expression is deregulated in the vast majority of human breast cancers.

Myc Expression in Primary Breast Tumors

Clinical studies on primary human breast cancer are valuable not only for predicting patient prognosis but also provide clues about tumor biology. It is generally agreed that there is an association between *c-myc* amplification and poor prognosis in breast cancer [discussed (23,24,30)]. An immunohistochemical study on Myc expression in breast tumors revealed that during tumor progression from well differentiated, low-grade to poorly differentiated, high-grade, Myc expression increased significantly (20). Very interesting in light of our results, described later, is the recent report that coamplification of *c-erbB2* and *c-myc* strongly correlates with a reduction in patient survival in a series of breast cancer patients (31). This suggests a certain level of cooperation between deregulation of these two proteins in breast tumor development.

How Does Myc Contribute to Breast Cancer Development?

Myc deregulation induces inappropriate proliferation, as evidenced by the ability of Myc to drive some quiescent cell lines into S-phase (12). This characteristic might contribute to cancer, not only by keeping cells cycling, i.e., preventing differentiation, but also by contributing to genomic instability. Deregulated Myc expression has been associated with gene amplification and karyotypic abnormalities in cultured cells (32,33) and with abnormal ploidy in *in vivo* models (34). Altered Myc expression accelerates the passage of cells through G1 into S-phase and has also been shown to allow cells to pass through mitotic checkpoints (35). Indeed, a comparison between A1N4 immortalized human mammary epithelial cells and A1N4 cells expressing exogenous Myc revealed that, while EGF-withdrawal caused a G1 arrest in both cell lines, EGF readdition induced premature entry into S-phase in the Myc-expressing cells (36). Additionally, expression of hTERT, a transcriptional target of Myc (37) has been demonstrated to be induced following ectopic expression of Myc in non-immortalized HMEC cultures. This is associated with increased telomerase activity in these HMECs, which may in part be responsible for the reported extended life-span of these cultures (37).

These characteristics of Myc have the potential to contribute to tumorigenesis by influencing cellular senescence and/or allowing cells with chromosomal

abnormalities or damaged DNA to replicate. Whether these effects reflect expression of very specific target genes, such as hTERT, or are due to overall effects on chromatin structure and ensuing gene expression, leading for example to chromosomal instability, remains to be elucidated (2).

Mouse Models for Myc-Induced Mammary Cancer

Transgenic mouse models have been developed in order to study the role of Myc in mammary transformation. Myc expression has been targeted to the mammary gland through the use of several specific promoters, including WAP and MMTV [reviewed (38,39)]. These mouse models have clearly demonstrated that deregulated Myc expression does indeed induce tumor formation in the mammary gland. However, in each Myc transgenic strain so far examined, the females display a long latency and a dependency upon passage through pregnancy for mammary tumor development. This characteristic is shared with most mammary-specific tumor models, reflecting the fact that a single oncogene is generally unable to induce a full tumor phenotype. The necessity for mice to pass through pregnancy before tumor development might be explained by the upregulation of the mammary specific promoters driving the transgene, as well as by the enhanced proliferation at this developmental stage, which may potentiate selection of additional mutations.

In order to test for oncogenic cooperativity, Myc transgenics have been crossed with mice expressing other oncogenes, including TGFα, Ha-Ras and Bcl-2. TGFα, a member of the EGF-related family of peptide growth factors, binds the ErbB1/EGF receptor and induces activation of EGF receptor dimers and ErbB2-containing heterodimers (40). In human breast cancer there is often coexpression of the EGF receptor and TGFα or one of the other EGF-related ligands, which leads to autocrine receptor activation (41,42). As observed for Myc transgenics, there was also a requirement for pregnancy and an extended tumor latency in the transgenic strains expressing TGFα in the mammary gland. In striking contrast, however, tumorigenesis in dual transgene carriers (coexpressing TGFα and Myc in the mammary gland) was dramatically enhanced (38,39,43–45). Furthermore, the double transgenic mice developed mammary tumors rapidly in all glands without a requirement for pregnancy or ovarian hormone stimulation (44). These results suggest that there is strong cooperativity between Myc and the ligand-activated EGF receptor in promoting mammary cancer.

Offspring arising from the cross of MMTV-Myc and MMTV-Ras transgenic strains also developed mammary tumors more rapidly than either of the single transgenic strains (46). Interestingly, tumor cells arising from MMTV-Myc mice showed a different cell cycle distribution in comparison to cells from tumors of the MMTV-Ras mice or the dual transgene carriers. In the Myc tumor cells there was a significant reduction in the G_1 fraction, compared to the cells taken from the Ras or dual transgene-induced tumors, and this was accompanied by an increase in the S-phase fraction (47). These observations accurately reflect the results seen in the A1N4-Myc cells discussed earlier (36), suggesting that mammary cells with deregulated Myc generally progress rapidly through G1.

As previously mentioned, Myc expression in the absence of growth factors often leads to apoptosis. Taking this in mind, the rapid tumor formation observed in the dual transgenics might in part be due to enhanced survival of Myc-expressing cells in glands with elevated levels of TGFα. In support of this proposition, examination of mammary tumors arising in double and single transgenics for apoptotic nuclei revealed that there was less apoptosis in the dual transgene carriers, coexpressing TGFα and Myc, than in Myc transgenics (43). Furthermore, when cells from the tumors coexpressing TGFα and Myc were cultured in the presence of an EGF receptor inhibitor, apoptosis was elevated (43). Tumors arising from transgenics coexpressing Myc and Ras also displayed a reduced rate of apoptosis when compared to Myc transgenics (47). These results suggest that deregulated Myc expression in the mammary gland promotes a high level of apoptosis. Only when pathways emanating from activated growth factor receptors are stimulated, is apoptosis reduced, allowing for the rapid onset of tumors.

Bcl-2, a pro-survival member of the Bcl-2 family, is able to inhibit apoptosis arising from a wide variety of insults, including deregulated Myc expression (48,49). Bcl-2 overexpression in the mammary gland, driven from a WAP-Bcl-2 transgene, was insufficient to induce mammary tumors (50). However, when these mice were crossed with Myc transgenics, accelerated development of Myc-induced tumor formation was observed. This was accompanied by a reduction in the fraction of apoptotic cells in the dual transgenic glands, when compared to glands expressing Myc alone (50). These results, as well as those discussed before, suggest that Myc's ability to induce

mammary cancer is enhanced upon suppression of its apoptotic activity. This can be achieved by various mechanisms, including increased expression of pro-survival members of the Bcl-2 family or increased activity of ErbB RTKs. However, it is quite likely that the cooperativity between Myc and TGFα goes beyond the ability of this peptide growth factor to suppress Myc-induced apoptosis. This supposition is based on the fact that tumor latency was shorter in the Myc/TGFα dual transgenics as compared to the Myc/Bcl-2 transgenics (44,50). This situation possibly relates to the observation that ErbB receptor activation impinges on multiple cellular pathways, whereas in comparison Bcl-2 has a relatively limited biological activity.

Equally relevant for this discussion are the phenotypes of dual transgenics coexpressing TGFα and ErbB2 in the mammary epithelium. These mice also display an accelerated rate of tumorigenesis in comparison to mice expressing a single transgene (51). Thus, co-expression of TGFα with either Myc or ErbB2 leads to accelerated tumorigenesis. It is, therefore, of great interest to examine the tumors arising in the TGFα-ErbB2 coexpressors. Based upon the results discussed later, showing that Myc is an effector of oncogenic ErbB2, one prediction would be that the mammary tumors arising in the dual TGFα-ErbB2 transgenics would have elevated levels of Myc expression. Taken together, these results from transgenic mice suggest that the mammary epithelium is particularly sensitive to deregulated expression of both ErbB RTKs and Myc; both of which are overexpressed in human breast cancer.

Myc as an Effector of Oncogenic ErbB2

Together with *c-myc*, amplification of the *c-erbB2* gene, leading to overexpression of the receptor, was one of the initial genetic alterations found in breast tumors (52,53). Today there is a wealth of clinical data demonstrating the importance of ErbB2, and other members of the ErbB family, in breast cancer. It is now clear that ErbB2 overexpression correlates with more aggressive tumor types and a worse patient prognosis (for reviews on this subject, the interested reader is referred to: 41,42,54,55). However, despite the obvious involvement of ErbB2 in breast tumor malignancy, the underlying mechanisms by which overexpression of this receptor potentiates tumor cell proliferation have remained poorly understood. In order to address the question of how ErbB2 overexpression contributes to the deregulated proliferation characteristics of tumors, we have used a strategy which allows efficient down-regulation of the receptor: intracellular expression of an ErbB2-specific single chain antibody (scFv-5R). Specific targeting of scFv-5R to the ER results in retention of ErbB2 in this compartment, leading to loss of receptor function (56). Using ErbB2-overexpressing SKBr3 breast tumor cells, inducible expression of scFv-5R was demonstrated to result in the loss of plasma membrane-localized ErbB2. Concomitant with this loss of functionally active ErbB2, there was a dramatic downregulation of ErbB3 activity and the MAP kinase and PI3K pathways, culminating in accumulation of the cells in the G1 phase of the cell cycle (11). Notably, the expression of Myc protein and mRNA was also decreased in the absence of ErbB2 signaling. Furthermore, ectopic expression of Myc partially overcame the scFv-5R-imposed G1 accumulation, indicating that Myc is a primary effector of ErbB2-mediated oncogenicity. A closer look at the mechanisms underlying reductions in Myc expression following ErbB2 downregulation, revealed that both Myc mRNA levels and protein stability were affected (11). As mentioned previously, the PI3K pathway is involved in the translational induction of Myc (28) and MAP kinase activation stabilizes the Myc protein (29). Taken together, therefore, our results suggest that enhanced ErbB2 signaling acts at multiple points to ensure that Myc levels remain elevated in breast tumor cells (Fig. 2).

Detailed analyses of cell cycle regulators in scFv-5R expressing SKBr3 cells revealed an important role for Myc in ErbB2-overexpressing cells. Specifically, the G_1 block induced by ErbB2 downregulation was demonstrated to be a consequence of redistribution of the CKI $p27^{Kip1}$ from sequestering complexes to cyclin E/Cdk2, resulting in cyclin E/Cdk2 inactivation. This redistribution was found to parallel the decrease in Myc and cyclin D protein levels (11). It is well established that the D-type cyclins as well as Myc play major roles in the regulation of $p27^{Kip1}$ complex formation (57). On the one hand, Myc stimulates D-type cyclin expression at the transcriptional level (21,22) and, on the other hand, the stability/activation of cyclin D-dependent Cdks is in turn facilitated by interaction with $p27^{Kip1}$ (57). It has also been proposed that other, as yet unknown, $p27^{Kip1}$ sequestration proteins may be downstream of Myc (58) ("x" in Fig. 2). Our results revealed that ectopic expression of Myc in scFv-5R expressing SKBr3 cells delayed the accumulation of these cells in G_1. This phenomenon correlated with enhanced cyclin D expression, inhibition of

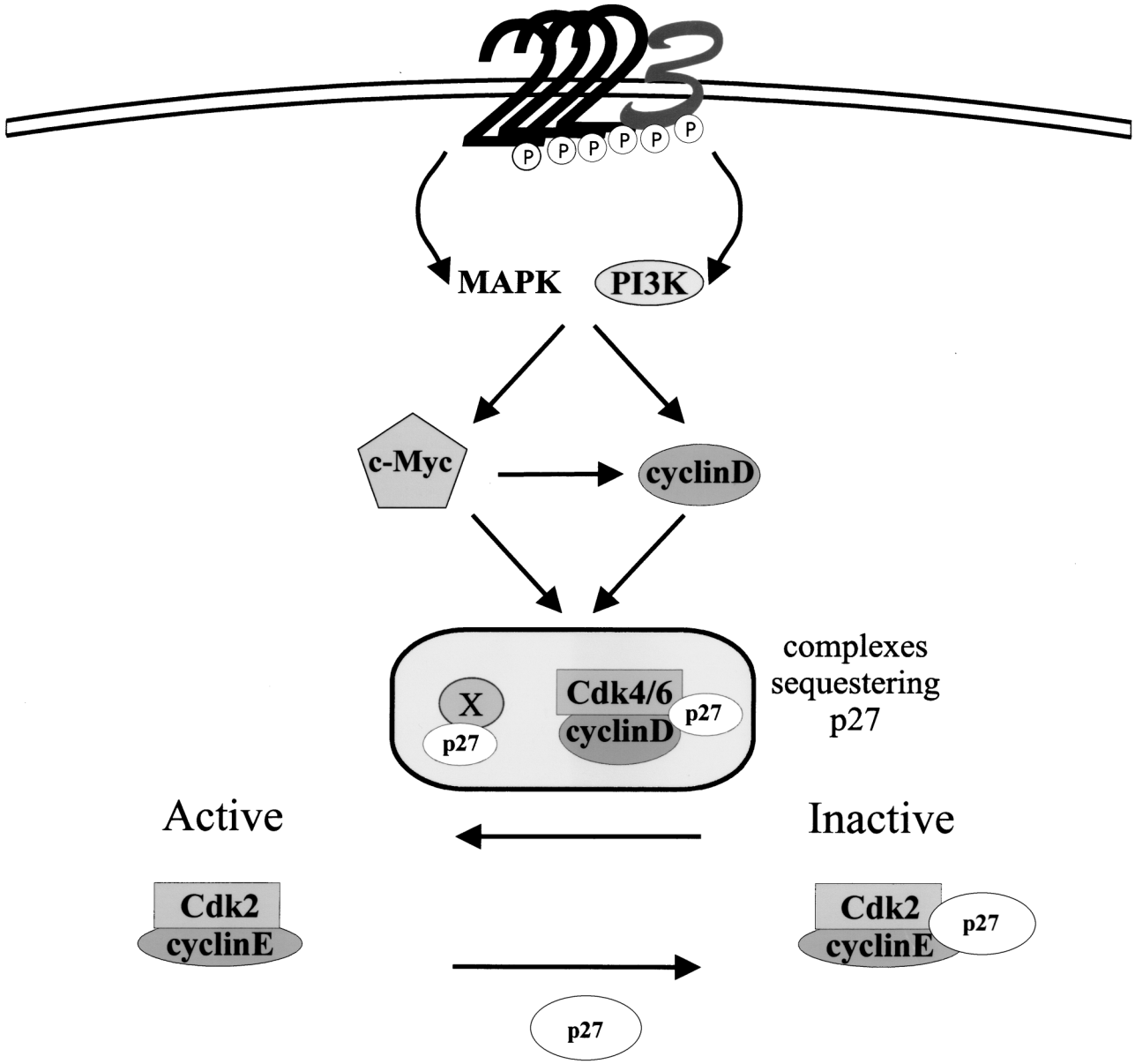

Fig. 2. A model of Myc as a downstream effector of oncogenic ErbB2. In breast tumor cells overexpressing ErbB2 there is activation of both ErbB2 and ErbB3, presumably resulting from concentration-dependent ErbB2 dimerization and lateral activation of ErbB3 (62). Dimers of ErbB2/ErbB3 are responsible for strong activation of the MAPK and PI3K cytoplasmic signaling pathways. Both pathways contribute to enhanced expression of the cell cycle regulators Myc and D-type cyclins, two proteins involved in $p27^{Kip1}$ complex formation. Upon loss of ErbB2 signaling, either following intracellular expression of scFv-5R or treatment of cells with mAb 4D5, the MAPK and PI3K cytoplasmic signaling pathways are down-regulated. This leads to a decrease in the level of Myc and the D-type cyclins. Myc stimulates D-type cyclin expression transcriptionally (21,22), however, the level of D-type cyclins is also controlled by the PI3K cytoplasmic signaling pathway (63). It has also been proposed that other, as yet unknown, $p27^{Kip1}$ sequestration proteins may be downstream of Myc (58) (indicated by "x"). Taken together, the drop in Myc and D-cyclin levels allows the redistribution of $p27^{Kip1}$ onto cyclinE/cdk2 and concomitant loss of kinase activity and G_1 block (see text for further details).

$p27^{Kip1}$ redistribution to cyclin E/Cdk2 complexes and maintenance of cyclin E/Cdk2 activity. These observations, therefore, add further weight to the proposal that elevated Myc activity plays a major role in ErbB2-dependent breast tumor proliferation, through the maintenance of $p27^{Kip1}$ sequestration proteins.

The clear importance of ErbB2 overexpression in the potentiation of breast tumor proliferation has resulted in intense scrutiny of ErbB2 as a target for tumor-directed therapies. In this respect, a mAb that targets the extracellular domain of ErbB2 (known as 4D5) specifically inhibits the *in vitro* growth of ErbB2-overexpressing breast tumor cells (59,60). Indeed, the humanized version of 4D5 (Herceptin™) has been validated in the clinic as an ErbB2-directed therapeutic approach (61). In order to understand the mechanism(s) underlying the effects of 4D5 on tumor cell growth, we set out to examine specific effects on cell cycle regulators in ErbB2-overexpressing breast tumor cells treated with this mAb (10). 4D5-mediated down-regulation of ErbB2 signaling led to a G1 accumulation in two ErbB2-overexpressing breast tumor cell lines (BT474 and SKBr3). Accumulation in G1 was preceded by a reduction in Cdk2 activity. Importantly, this correlated with a reduction in the expression of Myc and cyclin D proteins, as well as with an increase in $p27^{Kip1}$ association with cyclin E/Cdk2 complexes; events which were not observed with a noninhibitory control antibody (10). In BT474 cells, both Myc and D-type cyclin protein levels dropped rapidly (1–2 hrs) after 4D5 addition, with kinetics which slightly preceded the accumulation of the $p27^{Kip1}$ protein on cyclin E/Cdk2 complexes and cyclin E/Cdk2 inactivation. These data not only strongly support the previous observations outlined (11), but also point to the importance of ErbB2-dependent maintenance of Myc and cyclin D protein levels in ErbB2-overexpressing tumor cells in the clinical setting, particularly since coamplification of *c-erbB2* and *c-myc* correlates with poorer survival in breast cancer patients (31). Future experiments will be aimed at establishing the role of Myc in the deregulation of tumor cell proliferation; specifically as a downstream effector of elevated ErbB2 receptor activity.

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