Commentary

Inducible transgenics. New lessons on events governing the induction and commitment in mammary tumorigenesis

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Abstract

Breast cancer arises from multiple genetic events that together contribute to the established, irreversible malignant phenotype. The development of inducible tissue-specific transgenics has allowed a careful dissection of the events required for induction and subsequent maintenance of tumorigenesis. Mammary gland targeted expression of oncogenic Ras or c-Myc is sufficient for the induction of mammary gland tumorigenesis in the rodent, and when overexpressed together the rate of tumor onset is substantially enhanced. In an exciting recent finding, D'Cruz *et al* discovered tetracycline-regulated c-Myc overexpression in the mammary gland induced invasive mammary tumors that regressed upon withdrawal of c-Myc expression. Almost one-half of the c-Myc-induced tumors harbored K-*ras* or N-*ras* gene point mutations, correlating with tumor persistence on withdrawal of c-Myc transgene expression. These findings suggest maintenance of tumorigenesis may involve a second mutation within the Ras pathway.

Keywords: c-Myc, inducible transgenics, mammary oncogenes, Ras

Introduction

The advent of spatially and temporally regulated transgenics has allowed a careful dissection of the independent roles of oncogenes and tumor suppressors to the series of events governing initiation, progression and maintenance of tumorigenesis [1]. A surprising theme from these types of studies has been the finding that a single oncogene may drive tumor development, often with an invasive phenotype, but that withdrawal of the initiating oncogene may lead to complete tumor regression. The recent findings from the laboratory of Lewis Chodosh reveal that mammary-targeted expression of the c-Myc oncoprotein is sufficient for the induction of mammary tumorigenesis, and that withdrawal of the oncogenic stimulus results in tumor regression [2]. Previous studies with antisense oligonucleotides directed against c-*myc* mRNA (and other oncogenes) caused tumor regression, consistent with the model in which maintenance of tumorigenesis can be sustained through expression of a single oncogene. The rapid regression of invasive mammary tumors on withdrawal of mammary-targeted transgenic c-Myc expression [2] is consistent with a growing body of evidence that oncogene expression may be sufficient for the induction of but not the maintenance of tumorigenesis [3–6].

Oncogenic H-Ras (Val12→Gly12) under control of the reverse tetracycline transactivator targeted by the tyrosinase gene promoter in the $p16^{INK4+/-}$ background induces melanomas [4]. Tumors regressed on withdrawal of H-Ras (Val12→Gly12), although approximately one-third devel-

HMEC = human mammary epithelial cells; hTERT = catalytic subunit of the telomerase enzyme.

oped recurrence of phenotypically distinct neoplasms [4]. Targeting tamoxifen-inducible c-Myc expression to the suprabasal epidermis under control of the *Involucrin* promoter induced papillomatosis with angiogenesis that was reversible on withdrawal of c-Myc expression [6]. Higher levels of c-Myc expression correlated with more rapid development of the phenotype, and vascular endothelial growth factor production induced by c-Myc was postulated to contribute an *in vivo* paracrine survival signal [6]. Tetracycline-regulated c-Myc, targeted to the hematopoietic compartment under control of the immunoglobulin heavy chain enhancer, induced T cell lymphomas and acute myeloid leukemia that remitted on withdrawal of Myc expression [5]. In the 10% of mice in which relapse occurred, tumors had either escaped conditional regulation by the tetracycline system or had undergone a presumed secondary genetic event. Felsher and Bishop proposed that the genomic instability induced by c-Myc itself might have facilitated the accumulation of tumorigenic mutations [5].

Myc and collaborative oncogenesis

The current studies underscore the importance of collaborative oncogenes in Myc-induced tumorigenesis. In the studies by D'Cruz *et al*, tetracycline-regulated induction of c-Myc expression in the mammary epithelium induced mammary tumorigenesis rapidly, within 22 weeks, and with high penetrance (86%). The T_{50} value for mammary tumor occurrence in MMTV-Ha-Ras ($T_{50} = 168$ days) and MMTV-c-Myc ($T_{50} = 325$ days) was accelerated in the presence of both transgenes ($T_{50} = 46$ days) in previous *in vivo* studies [7]. Furthermore, the Ras transgene abrogated the requirement for pregnancy in c-Myc-induced mammary tumor onset [7,8]. Myc collaborates with a variety of signaling pathways, including Ras, Bcl-2, a transcriptional repressor in the polycomb family (Bmi-1), Notch, *twist*, and factors that inactivate p53. Suppression of apoptosis enables oncogenes such as c-*myc* and E1A to acquire full oncogenic activity [9]. In primary mouse embryo fibroblasts, c-Myc-induced apoptosis requires CD95 (Fas/APO-1), Apaf-1 and Caspase 9. Insulin-like growth factor-1 signaling and Bcl-2 suppress Myc-induced apoptosis, and *bcl-2* overexpression suppressed c-Myc transgene-induced apoptosis and accelerated mammary tumorigenesis. Since Myc induces apoptosis, it has been predicted that collaborative oncogenesis may occur with pathways that selectively inactivate Myc-induced apoptosis. For example, c-Myc-induced apoptosis is dependent on the induction of 19^{ARF} [10], and the strong collaboration in oncogenesis between the Eμ-Myc and Bmi-1 involves the downregulation of INK4A/ARF. Several different types of collaborative interactions between Ras and Myc are known. Ras fails to inhibit Myc-induced apoptosis in fibroblasts, although growth factor rescue of c-Myc-induced apoptosis may occur in epithelial cells through the Ras-mediated induction of Akt phosphorylation [11–13]. Myc is induced by proliferative signals, and the serum-induced increase in the Myc half-life is mediated by Ras activation through Raf/ERK and Akt pathways [14].

How might oncogenic Ras contribute to ongoing tumorigenesis? The important and exciting new observation made by D'Cruz *et al* [2] was that almost one-half of the c-Myc-induced mammary tumors harbored point mutations in the K-*ras* or N-*ras* gene. Furthermore, there was a significant correlation between the presence of point mutations in the *ras* gene and the persistence of tumorigenesis following downregulation of transgenic c-Myc expression, suggesting that maintenance of mammary tumorigenesis involves a second mutation with the Ras pathway. Oncogenic Ras is known to contribute both cell nonautonomous tumor–host interactions and cell autonomous activities. Ras may confer the ability to evade the host immune response and sustain tumor cell vasculature through regulating vascular endothelial growth factor expression. The maintenance of tumor growth by H-Ras (Val12→Gly12) in an inducible *in vivo* melanoma model suggested a role for both cell autonomous functions of Ras in tumor maintenance and a role for the induction of vascular endothelial growth factor expression. However, this was not sufficient to overcome tumor regression [4]. Transformation by Ras can only proceed in the presence of additional mutations that prevent Ras-induced senescence. Myc and E1A inactivate cellular responses that are required for Ras-mediated inhibition of cellular proliferation.

Human mammary epithelial cells and maintenance of transformation.

What is the relevance of these studies to human breast cancer? The majority of human breast cancers also arise from mammary epithelia, and c-*Myc* is found amplified (15%), rearranged (5%) and overexpressed in about 70% of cases, suggesting an important role in genesis or progression of breast cancer [1,15]. Signaling via the retinoblastoma protein (pRB) pathway is frequently inactivated either through overexpression of cyclin D1 or loss of expression of either the pRB or $p16^{Ink4a}$. Mutations in the p53/ARF tumor suppressor pathway occur in up to one-half of all tumors. Alterations in the Ras signaling pathway, mediated through amplification or overexpression of the ErbB2/Neu gene, are found in up to 30% of patients. This perhaps explains the low frequency of *ras* gene mutations found in human breast cancers. At a more fundamental level, however, rodent cells can be transformed through concomitant expression of two oncogenes, whereas immortalization of human epithelial cells has additional requirements [16]. Myc is sufficient for the partial transformation of both mouse and human mammary epithelial cells, growing on soft agar in response to epidermal growth factor. Human fibroblasts [16] or human mammary epithelial cells (HMEC) can be transformed through the

coexpression of oncogenic Ras (H-*ras*V12), the SV40 large T and the catalytic subunit of the telomerase enzyme (hTERT) [17]. Consistent with the importance of Ras found in the studies from the laboratory of Lewis Chodosh, an important correlation was previously found between the level of Ras oncogene expression and the tumorigenicity of the HMEC. Intriguingly, the HMEC transformed in this manner expressed amplified c-Myc [17], emphasizing an important inter-relationship between Ras and c-Myc in the transformation of HMEC. An intriguing question remains as to what role hTERT is playing in in human breast cancer. hTERT is the catalytic subunit of the telomerase enzyme. HMEC that emerge from senescence exhibit eroding telomeric sequences and ultimately enter telomere-based crisis to generate the types of chromosomal abnormalities seen in the earliest lesions of breast cancer [18]. hTERT overexpression in human cells prior to senescence induces indefinite cellular proliferation, although there may not always be a corresponding lengthening of the telomeres [19].

How might Myc overexpression contribute to the induction of mutations within the *ras* gene? The current studies by D'Cruz *et al* identify another potentially important mechanism for collaboration between c-Myc and Ras, and raise the strong possibility that these events may be required for the maintenance of rather than simply induction of tumorigenesis: the induction by c-Myc of activating mutations within the K-*ras2* gene. The high prevalence of the *ras* gene mutations and the identification of similar mutations in other models of c-Myc-induced mammary tumorigenesis suggest c-Myc overexpression may be causal in these mutagenic events. The induction of Ras mutations may be a consequence of c-Myc-induced genomic instability. c-*myc* and several other oncogenes have been shown to play a role in driving genomic instability [20,21]. Spectral karyotyping has revealed that chromosomal abnormalities are common in c-Myc-induced lymphomas and that transient c-Myc expression is sufficient for the induction of genomic instability. c-Myc may contribute to genomic instability by shortening the G1 to S phase transition. The mechanism by which c-Myc triggers G1 exit is in part through promoting increased cell mass, enhancing G1 cyclin/CDK activity, and by negatively modulating abundance [22] and function [23] of the cell cycle inhibitor $p27^{Kip1}$. Forcing cells through the cell cycle in the presence of local DNA damage may inhibit expression of genes involved in cell-cycle transition, or may titrate co-activators that normally subserve functions regulating genomic stability. The role of c-Myc-induced telomerase activity in regulating genomic instability is unclear at this time. Ectopic expression of c-Myc activates telomerase in HMEC [24,25] and, conversely, hTERT overexpression selects for c-Myc overexpression that may be anticipated to thereby contribute to genomic instability [24,25]. Surprisingly, hTERT-transduced HMEC overexpressing c-Myc had a normal chromosome number. These findings together suggest that, although capable of extending the life span of HMEC, hTERT is not 'genoprotective'.

Conclusions

The past decade has seen important progress in the development of temporally regulated transgenics. The careful molecular dissection of the events regulating myc-dependent multistep mammary tumorigenesis, by the laboratory of Lewis Chodosh, has uncovered another important insight into the events governing commitment to tumorigenesis. The finding that activation of the Ras pathway and overexpression of c-Myc play a role in tumor commitment suggests that therapeutics targeting multiple pathways may be beneficial. It is known that c-Myc inhibits cellular differentiation, enhances progression through the G1 phase of the cell cycle, activates apoptosis, induces anabolism, increases genomic instability and increases telomerase activity [26]. Important biological activities of c-Myc require Myc Box II, the deletion of which abrogates transrepression function, cell-cycle progression, cofactor TRRAP binding and transformation in cultured cell systems. The use of transgenics targeting c-Myc mutants proficient in cell-cycle progression but deficient in apoptosis [27] may provide important insights into the role of apoptosis in c-Myc-induced tumor onset and progression. Identifying the molecular mechanisms by which c-Myc contributes to the separable functions of tumor induction versus maintenance will be of great interest in the future. The studies of D'Cruz *et al* suggest that determining the mechanisms by which c-Myc regulates genomic instability may help us to understand events leading to irrevocable commitment to mammary tumorigenesis.

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