A Dominant Mutant Allele of the ING4 Tumor Suppressor Found in Human Cancer Cells Exacerbates MYC-Initiated Mouse Mammary Tumorigenesis

Suwon Kim1,2,3, Alana L. Welm1,4, and J. Michael Bishop1

Abstract

ING4 is a candidate tumor suppressor gene that is deleted in 10% to 20% of human breast cancers and is mutated in various human cancer cell lines. To evaluate whether ING4 has a tumor-suppressive role in breast tissue, we overexpressed it in mouse mammary glands using a transplant system. Ectopic expression of ING4 suppressed MYC-induced mammary hyperplasia, but not tumorigenesis. In the same model system, we show that a COOH-terminal truncation mutant of ING4 found in human cancer cells could act alone to induce abnormal gland structures resembling mammary hyperplasia, which did not progress to tumors. However, coexpression of the ING4 mutant with MYC increased the penetrance and metastasis of MYC-initiated mammary tumors, giving rise to tumors with more organized acinar structures. Similarly, in vitro expression of the ING4 mutant in MCF10A mammary epithelial cells reinforced tight junctional structures. Our results provide direct functional evidence that ING4 could suppress the early stages of breast cancer and that dominant mutant alleles of ING4 might contribute to malignant development.

Introduction

ING4 is a member of the inhibitor of growth (ING) tumor suppressor family and has been shown to play a role in various cancer-related cellular processes, including cell cycle regulation, apoptosis, DNA damage response, cell migration, contact inhibition, and tumor angiogenesis (1–8). To date, five members (ING1–5) have been identified in the ING family, each of which contains a conserved plant homeodomain (PHD) finger motif within the COOH-terminal half of the proteins (9, 10). PHDs are composed of 50– to 80–amino acid residues containing a Cys4-His-Cys3 motif that coordinates the binding of two Zn2+ molecules (9, 11). PHD fingers are found in nuclear proteins that modulate transcription via chromatin remodeling (11). Consistent with a role in chromatin remodeling, the ING family members form complexes with histone acetyltransferases and histone deacetylases (12). ING4, in particular, copurifies with histone acetyltransferases such as p300 and HBO1 (1, 12). Furthermore, the PHD of ING4 could directly bind to trimethylated histone H3 at lysine 4 (H3K4me3; refs. 13–15), suggesting more than one mode of association with chromatin. Recently, Hung and colleagues, (15) showed that a mutation in the PHD domain abolished both gene regulation and suppression of cellular transformation in vitro by ING4, thus demonstrating a requirement for the PHD domain in ING4 function.

ING4 has been shown to modulate transcription factors implicated in cancer, such as p53, NF-κB, and hypoxia-inducible factor 1-α (1, 5, 7, 16, 17). Although ING4 could enhance transcription depending on the p53 tumor suppressor in colorectal cancer cells (1), ING4 suppressed the transcription of NF-κB target genes implicated in tumor phenotypes in glioma (5, 16). These results support the notion that the gene regulation activity of ING4 is directly related to the tumor suppressor function of ING4.

ING4 function is disabled in human cancers by multiple mechanisms. Loss of heterozygosity and single-locus deletion of ING4 have been found in head and neck squamous cell carcinoma (18) and breast cancer (4), respectively. Downregulation of ING4 expression correlates with high-grade tumors and poor patient outcome in cancers, including glioma, melanoma, gastric adenocarcinoma, and hepatocellular carcinoma (5, 16, 19–21). In addition, mutations of ING4 have been identified in gliomas (16) and several cancer cell lines (4). In xenograft studies, ING4 has been shown to suppress tumor growth of glioma (5) and lung cancer cells (22). Thus, it seems that ING4 may serve as a tumor suppressor in a variety of tissues.

We previously reported that ING4 was among the genes that emerged from an in vitro screen for potential tumor suppressors, using loss of contact inhibition as an indicator (4). In addition, we found that ING4 was deleted in 10% to 20% of breast cancers, suggesting a role for ING4 as a tumor suppressor in breast cancer (4). We now report that ING4 could
suppress an initial hyperplastic response to the oncogene MYC in a mouse model of breast cancer. In addition, we show that a COOH-terminal truncation mutant of ING4 found in a human neuroblastoma cell line induced mammary hyperplasia and exacerbated MYC-initiated mammary tumorigenesis. These results sustain the view that ING4 could function as a tumor suppressor gene in breast tissue, and the reciprocal conclusion that a dominant mutant allele of the gene might contribute to some forms of tumorigenesis in humans. In either instance, the effect of the gene seems to be early in tumorigenesis.

**Materials and Methods**

**Retroviral transduction of primary mouse mammary epithelial cells**

ING4 and ING4mt14 (nt465ΔC; ref. 4) were cloned into the pMIG (pMSCV-IRES-GFP) retroviral vector using an EcoRI restriction enzyme site upstream of the IRES sequence (4). MYC was cloned into the pMICD8 (pMSCV-IRES-CD8) retroviral vector using an EcoRI restriction enzyme upstream of the IRES sequence. pMIG and pMICD8 vectors were generously provided by Dr. Yosef Refaeli (National Jewish Medical Research Center, Denver, CO). Retroviral production and infection were done according to the methods described by Welm and colleagues (23). In brief, the retroviral particles were produced by transfecting BOSC ecotropic packaging cells with pMIG, pMIG/ING4, pMIG/ING4mt14, or pMICD8/MYC. The supernatant containing the virus was used to infect primary mouse mammary epithelial cells (MEC) in tissue culture, prepared from 10- to 12-week-old FVB female mice. The infection rate was determined by fluorescence-activated cell sorting analysis for green fluorescent protein (GFP) and/or CD8-positive cells.

**MEC transplantation and analysis of reconstituted mammary glands and tumors**

One million gene-transduced MECs were surgically transplanted into the cleared mammary fat pads of 3-week-old syngeneic recipient mice. The reconstituted mammary glands were examined 8 to 10 weeks after transplantation using a fluorescent dissecting microscope. Tumor diameter was measured weekly with a caliper. When tumors reached a diameter of 2.5 cm, the mice were sacrificed for postmortem examination and harvest of tumors and organs including axillary lymph nodes, kidney, liver, spleen, thymus, and lungs. Tumors and organs were fixed in paraformaldehyde (4% in PBS) at 4°C for 4 to 16 hours before processing for histology.

**Immunohistochemistry and immunofluorescence**

Immunohistochemical staining of tumor sections was performed using ABC Elite and MOM immunodetection reagents according to the recommendations of the manufacturer (Vector Laboratories). Antibodies used were anti–Ki67 rabbit polyclonal (1:1,000; Zymed), anti–E-cadherin mouse monoclonal (1:1,000; BD Biosciences), and anti–Zo-1 rabbit polyclonal (1:1,000; Zymed). Secondary antibodies used for immunofluorescence were rhodamine-conjugated goat anti-mouse (1:1,000) and donkey anti-rabbit (1:10,000) antibodies purchased from Jackson ImmunoResearch Laboratories. For apoptosis staining on tumor sections, ApopTag Peroxidase kit (Millipore) was used as described by the manufacturer. Proliferation and apoptosis were measured by counting Ki67-positive cells and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL)-positive cells, respectively, in a given microscopic field, and calculating the percentage of stain-positive cells compared with the total number of cells. Three or more independent fields were evaluated to obtain the average value.

**Results**

**ING4 suppresses MYC-induced mammary hyperplasia in mice**

Our previous work raised the possibility that ING4 might serve as a tumor suppressor in breast tissue (4). To investigate this hypothesis further, we used a mouse model of breast cancer that uses transplantation of mouse MECs into which genes have been transduced by a retroviral vector (23, 25). In the present work, we used the pMICD8 and pMIG retroviral vectors to transduce MYC and ING4, respectively, into primary MECs harvested from 8- to 10-week-old FVB female mice. The ING4 construct coexpressed GFP and the MYC construct coexpressed human CD8. We achieved an average of 20% to 25% MECs infected with virus, as assessed by using fluorescence-activated cell sorting to quantify GFP+ and CD8+ cells. Of those that were infected, >90% were GFP+CD8+ double-positive (data not shown). A million cells (~18% GFP+CD8+, 1% GFP-, and 1% CD8+) were surgically transplanted into the cleared mammary fat pads of 21-day old syngeneic FVB female mice. The reconstituted glands were dissected 8 weeks after transplantation and visualized by fluorescent microscopy.

Reconstitution of mammary glands upon transplantation requires functional mammary stem/progenitor cells (26). MECs infected with the control vector expressing GFP alone gave rise to full outgrowths of normal but GFP-negative mammary glands (Fig. 1A and B), despite the fact that we transplanted ~200,000 GFP+ cells. We concluded that mammary stem/progenitor cells were rarely infected by the retroviral vector, consistent with previous findings (25). Transplantation of cells expressing ING4/GFP yielded a comparable result (data not shown).

In contrast, cells coexpressing MYC/CD8 and GFP reconstituted GFP-positive mammary glands >90% of the time (19 out of 21 glands; data not shown). This result suggested that MYC overexpression might provide a proliferative
hyperplastic lobuloalveolar growths were often visible (arrows in Fig. 1G–H). These data show that ING4 could suppress MYC-induced mammary hyperplasia, suggesting a role for ING4 as a tumor suppressor in the early stages of breast cancer.

It is noteworthy that MECs coexpressing MYC/CD8 and ING4/GFP reconstituted GFP+ mammary glands that filled the fat pads (Fig. 1E), indicating that ING4 expression did not adversely affect the proliferative advantage of stem or progenitor cells that overexpress MYC, or the ability of MYC to transform cells into stem/progenitor-like cells. These results suggest that ING4 might suppress Myc activity only in more differentiated epithelial cells, allowing proliferative expansion of the stem/progenitor cells overexpressing MYC.

We next examined mammary tumor frequencies in mice that were transplanted with cells transduced with either MYC or MYC/ING4. In our previous report, transduced MYC elicited mammary hyperplasia, but not tumors, in 4 months (23). In this study, we observed tumors in mice transplanted with MYC-expressing cells, by virtue of observing the animals for a longer period of time (7 months). The tumor frequencies were comparable for mice transplanted with cells expressing MYC or MYC/ING4: 44% of mice grafted with cells expressing MYC developed tumors, and 40% of mice grafted with cells expressing MYC/ING4 developed tumors (Fig. 2C).

There was no change in tumor latency (Fig. 2C).

The failure of ING4 to suppress tumorigenesis by MYC could be attributable to the incomplete suppression of hyperplasia by ING4 noted above. Alternatively, spontaneous events might occur that eventually circumvent the effect of ING4 during the course of tumorigenesis (see Discussion).

Expression of an ING4 truncation mutant induces mammary hyperplasia and cooperates with MYC in mammary tumorigenesis in mice

In a previous study, we identified mutations in ING4 transcripts isolated from various cancer cell lines (4). We investigated the activity of one of the mutations in the mouse mammary gland. The mutation in question was found in a neuroblastoma cell line and is a single nucleotide deletion at nucleotide position 465 that results in a frame shift leading to a truncation of the COOH-terminal half of the ING4 protein (Fig. 2A). The frame shift results in the addition of six nonnative amino acids (RLPRRS) after the residue Pro155 (Fig. 2A). We will refer to this mutant allele as ING4-mt14.

We overexpressed ING4-mt14 in mouse mammary glands using the transplant system described above. In comparison with the glands reconstituted with vector-infected cells, ING4-mt14 expressing glands displayed hyperplastic features, including increased number of lobuloalveolar structures and multiple budding that was more pronounced in quaternary ducts (Fig. 2B, c–d). In contrast, MYC overexpression induced lateral budding and multiple branching that was visible in all ducts in addition to hypergrowth of lobuloalveoli (Fig. 2B, e–f). Histologic examination of ING4-mt14 expressing glands at 8 weeks posttransplant showed increased number of ductal acini per section but no abnormalities in the number of epithelial layers or in the organization of the layers (data not shown).
When both ING4mt14 and MYC were expressed simultaneously, the hyperplastic phenotype became more severe and the individual ductal branching points were difficult to discern (Fig. 2B, g–h). Glands expressing MYC or MYC/ING4mt14 progressed to mammary tumors, whereas glands expressing ING4mt14 alone did not develop tumors over a period of 7 months (Fig. 2C). Mice were followed for a period of 12 months and no tumors were detected (data not shown).

MYC-initiated hyperplastic mammary glands progressed to mammary tumors with ~40% penetrance over a period of 1 year, with an average tumor latency of 12 weeks (Fig. 2C). When both MYC and ING4mt14 were expressed, tumorigenesis was accelerated and penetrance was increased to >90% (Fig. 2C). These data show cooperation between MYC and ING4mt14 in mouse mammary tumorigenesis. By measuring tumor diameters over the course of time, we examined whether tumors generated by coexpressing MYC and ING4mt14 grew faster than tumors expressing only MYC. The results showed that the tumors grew at a comparable rate once they appeared (Fig. 2D). We conclude that ING4mt14 is a dominant allele that might contribute to MYC-initiated mammary tumorigenesis early in its course, resulting in increased tumor penetrance with a shortened latency, but not during the stage of tumor growth (see Discussion).

Figure 2. A truncation mutant of ING4 (ING4mt14) induces hyperplastic lobuloalveolar structures and exacerbates MYC-initiated mammary tumorigenesis. A, schematic diagram of ING4 wild-type and ING4mt14 (LID, Lamin interaction domain; NLS, nuclear localization signal; PHD, plant homeodomain). A single nucleotide deletion (465delC) results in a truncation of the protein at amino acid 155 and the addition of six nonnative amino acids RLPRRS. B, a and b, glands reconstituted with GFP expressing cells (whole mount); c and d, ING4mt14/GFP expressing glands; e and f, MYC/GFP; g and h, MYC/ING4mt14/GFP; C, percentage of tumor-free mice 27 wk posttransplant (serrated line, ING4, \( n = 17 \); solid line, MYC, \( n = 27 \); dotted line, MYC/ING4, \( n = 10 \); uneven serrated line, MYC/ING4mt14, \( n = 31 \)). D, tumor growth, measured as average tumor diameters at each time point (open square, MYC, \( n = 7 \); solid square, MYC/ING4mt14, \( n = 9 \)).
Tumors generated by coexpressing MYC and ING4mt14 display increased metastasis

We used the expression of GFP in transduced cells to visualize metastasis to distant organs including lymph nodes, lung, spleen, and thymus (Fig. 3A–E). Tumors expressing MYC alone or MYC/ING4mt14 metastasized to lymph nodes at comparable frequencies of 35% and 30% (Fig. 3F, second column). However, MYC/ING4mt14 tumors metastasized to lungs at a 5-fold higher frequency compared with tumors expressing MYC alone (55% versus 10%; Fig. 3F, first column). In addition, we observed metastasis of tumors expressing MYC/ING4mt14 to other organs including the thymus and spleen (Fig. 3D, E, and F, third and fourth columns). We conclude that ING4mt14 not only cooperates with MYC early in tumorigenesis, but also provides a metastatic advantage to the tumor cells.

Tumors that coexpress MYC and ING4mt14 are less proliferative and less apoptotic

The cells in tumors elicited by either MYC or MYC/ING4mt14 were cytokeratin-18 positive, indicating origination from the luminal epithelial cell lineage (data not shown). MYC tumors were mostly papillary in nature and were packed with cells containing large nuclei (Fig. 4A). In comparison, MYC/ING4mt14 tumors appeared more structurally organized with visible acinar structures (Fig. 4F). Similar structural organization was also apparent in lymph node and lung metastases from MYC/ING4mt14 tumors (data not shown).

We measured the proliferation of tumor cells by staining tumor sections with an antibody against Ki67. In MYC tumors, >80% of cells were Ki67-positive, revealing the highly proliferative nature of the MYC-transformed tumor cells (Fig. 4B). In contrast, <20% of cells in MYC/ING4mt14 tumors were positive for Ki67 (Fig. 4G).

We then compared the number of apoptotic cells in the two types of tumors. We detected >20% apoptotic cells in MYC tumors (Fig. 4C), whereas <5% of MYC/ING4mt14 tumor cells seemed to be apoptotic (Fig. 4H). Therefore, the ratio between proliferative cells and apoptotic cells was roughly the same in both types of tumors (4:1), in accord with the comparable growth rates between MYC and MYC/ING4mt14 tumors (Fig. 2D). We conclude that coexpression of ING4mt14 might render the MYC tumor cells less proliferative and less apoptotic.

Tumors that coexpress MYC and ING4mt14 are structurally more organized and show apical tight junction staining

One of the features that distinguished MYC/ING4mt14 tumors from MYC tumors was more organized glandular structures (Fig. 4A and F). To explore the structural organization of the tumors, we immunostained for the adherens junction marker E-cadherin and the tight junction marker ZO-1. E-cadherin was localized to the membranes between adjoining cells in both MYC and MYC/ING4mt14 tumors (Fig. 4D and I). In contrast, there was a striking difference in ZO-1 staining between the two types of tumors (Fig. 4E and J). Although MYC tumors displayed diffuse and some punctate staining, MYC/ING4mt14 tumors had distinct tight junctions, localized to the apical lateral cell membranes within the acinar structures, suggesting an organization similar to that found in normal mammary ducts. We also observed the same staining pattern with another tight junction protein, occludin-1 (data not shown). Therefore, it seems that ING4mt14 expression in the tumors results in the retention or re-establishment of tight junction structures that contain ZO-1 and occludin.

ING4mt14 expression establishes tight junction strands in MCF10A tissue culture cells

We next explored whether the expression of ING4mt14 could elicit the cellular features we observed in MYC/ING4mt14 tumors in tissue culture. We used MCF10A cells,
which are immortalized basal-like human MECs with no discernible tight junctions in culture (28). We expressed vector, \textit{ING4}mt14, \textit{MYC}, or \textit{MYC}/\textit{ING4}mt14 in MCF10A cells. We observed no differences in the growth rate or apoptosis between cells expressing the various constructs (data not shown). We then stained for ZO-1. In cells expressing vector or \textit{MYC} alone, ZO-1 staining was diffuse and cytoplasmic with occasional membrane staining (Fig. 5A and B). Expression of wild-type \textit{ING4} resulted in the similar ZO-1 staining as the vector or \textit{MYC} alone (data not shown). When \textit{ING4}mt14 was expressed, more organized membrane staining of ZO-1 was visible (Fig. 5C). When both \textit{MYC} and \textit{ING4}mt14 were expressed in cells, more contiguous and numerous tight junction strands were visible between the cells (Fig. 5D). We conclude that expression of \textit{ING4}mt14 alone could induce tight junction structures in tissue culture. Moreover, coexpression of \textit{MYC} seems to augment the induction of tight junctions by \textit{ING4}mt14. Taken together with the observation that tumors generated by coexpressing \textit{MYC} and \textit{ING4}mt14 also showed distinct tight junction structures \textit{in vivo}, we conclude that \textit{ING4}mt14 might exacerbate \textit{MYC}-initiated tumorigenesis in part by establishing tight junction structures.

**Discussion**

**ING4 functions in the early steps of mammary tumorigenesis**

We have found that \textit{ING4} could suppress \textit{MYC}-induced mammary hyperplasia in a mouse model of breast cancer. The mechanism of this suppression is not known. Given the apparent ability of the \textit{ING4} protein to regulate transcription factors, however, it is possible that \textit{ING4} modulates the transcriptional activity of the \textit{MYC} gene product. Although we could not show direct binding between the \textit{ING4} and \textit{Myc} proteins, we cannot rule out the possibility that a complex containing both proteins exists \textit{in vivo}. Paradoxically, \textit{ING4} could not suppress \textit{MYC}-initiated tumorigenesis in our transplant system, as determined by tumor penetrance and latency. It might be that one or more events during the course of tumor progression circumvent suppression by \textit{ING4}.

We have found that a truncation mutant of \textit{ING4} could induce morphologic abnormalities resembling mammary hyperplasia on its own. Coexpression of the \textit{ING4} mutant exacerbated \textit{MYC}-initiated mammary tumorigenesis by decreasing tumor latency and increasing tumor penetrance and metastasis. These results suggest that the \textit{ING4} mutant may be a dominant negative allele that interferes with the tumor-suppressive function of the wild-type allele and contributes to tumorigenesis. We found the \textit{ING4}mt14 allele in a neuroblastoma cell line, and a similar mutation that results in a COOH-terminal truncated \textit{ING4} in a lung cancer cell line (4) and glioma cells (16). We have not examined whether an analogous mutation ever occurs in human breast cancer, although our results raise the possibility that such a mutation could contribute to tumorigenesis in the human breast.

\footnote{S. Kim, unpublished results.}
Our findings suggest that ING4 acts at an early step in breast tumorigenesis. First, the wild-type allele could suppress the induction of hyperplasia by MYC, but ultimately fails to attenuate tumorigenesis. Second, a dominant mutant allele of the gene elicits abnormal gland structures resembling hyperplasia when acting alone, but not full tumorigenesis. Third, the mutant allele accelerates and increases the penetrance of tumorigenesis elicited by MYC, but has no effect on the rate at which established tumors grow. By the same reasoning, the ability of the mutant allele to augment tumor metastasis would suggest that metastatic potential of tumor cells could be set relatively early during tumor progression.

A dominant mutant allele of the ING4 tumor suppressor

The dominant activity described here for ING4mt14 conforms with previous findings with well-established tumor suppressor genes. For example, dominant negative alleles of TP53 have been described in many different types of human cancer. These alleles encode inactive proteins that bind to the wild-type protein to create inactive hetero-oligomers (29). The p63 and p73 tumor suppressors, homologues of p53, also have dominant negative forms that lack an NH2-terminal transcription activation domain. These ΔN isoforms were shown to interfere with the activity of the wild-type p63 and p73 proteins as well as that of p53 (30).

We have no evidence that ING4 functions as a homologomer. Therefore, direct binding of ING4mt14 to the wild-type ING4 protein creating inactive hetero-oligomers seems unlikely. It is more likely that ING4mt14 competes for factors that normally bind to and are required for the function of the wild-type ING4 protein.

The mutation in ING4mt14 results in the deletion of amino acids 156–180, which are a part of the nuclear localization signal (amino acids 129–180, see Fig. 2A). However, we localized a hemagglutinin epitope–tagged ING4mt14 protein to the nucleus in MCF10A cells (immunostaining with anti-HA antibody; data not shown). Thus, improper localization of the mutant protein, and consequently, of ING4 binding partners, does not seem to be the mechanism by which ING4mt14 carries out its aberrant function. Alternatively, ING4mt14 might compete with the wild-type protein for binding to the nuclear scaffold protein, Lamina A. A conserved Lamin interaction domain located in the NH2-terminal half of the ING family proteins has recently been described (31). When the Lamin interaction domain of ING1 was overexpressed, the wild-type ING1 was displaced from the nucleus, rendering ING1 nonfunctional (31). We tested this possibility by coexpressing ING4mt14 and a hemagglutinin epitope–tagged wild-type ING4 and found that wild-type ING4 was localized to the nucleus (data not shown). Therefore, ING4mt14 does not seem to displace the wild-type protein from the nucleus.

ING4mt14 retains amino acids 141–145 in nuclear localization signal, which were shown to facilitate binding to p53 (32). Thus, it is possible that ING4mt14 functions as an oncogene by directly interfering with the p53 tumor suppressor function and/or interfering with the wild-type ING4 function that enhances the p53 activity. We found that transplantation of p53-null MECs form mammary tumors with 100% penetrance, whereas ING4mt14-expressing cells reconstituted hyperplastic glands but did not progress to tumors. In addition, a p53 heterozygous-null genetic background did not increase MYC-initiated tumor penetrance or metastasis. Therefore, the molecular mechanism of ING4mt14 in exacerbating MYC-initiated mammary tumorigenesis does not seem to be via inactivation of p53.

The domain that is entirely missing in ING4mt14 is PHD, which is required for the function of ING4 (15). PHD of ING4 was shown to facilitate binding of ING4 to both methylated histone H3 (13, 14) and HPH2 (hypoxia-inducible factor prolyl hydroxylase 2), a regulatory subunit of hypoxia-inducible factor 1-α (17). Although it is clear that ING4mt14 has lost the wild-type function, it seems unlikely that the inability to bind to H3K4me3 or HP1H2 would exert a dominant negative effect. Rather, ING4mt14 binding to factors necessary for the wild-type ING4 function might result in a dominant negative effect. Binding partners for the NH2-terminal portion of the ING4 protein are currently unknown. These partners might provide a platform to elucidate the dominant negative mechanism of ING4mt14.

ING4mt14 exacerbates MYC-initiated mouse mammary tumorigenesis

Mammary tumors generated by coexpressing MYC and ING4mt14 occurred at a higher frequency and were more metastatic, compared with the tumors generated by expressing MYC alone. In gliomas, ING4 is shown to inhibit NF-κB/IL-8 induced tumor angiogenesis, thus suppressing tumor growth (5). We examined whether ING4mt14 affects tumor angiogenesis by immunostaining tumors for an endothelial cell marker, CD31. We did not see a discernible difference in either the intensity or distribution of CD31 staining between MYC tumors and MYC/ING4mt14 tumors (data not shown). Consistent with this observation, the levels of IL-8 or CD31 transcripts were comparable in the two types of tumors, when determined by quantitative reverse transcription PCR (data not shown). Therefore, it seems unlikely that enhanced tumor angiogenesis provides an explanation for the ability of ING4mt14 to exacerbate MYC-induced mammary tumorigenesis.

MYC/ING4mt14 tumors appeared more structurally organized and acinar structures were also discernible in lymph nodes and pulmonary metastases of the MYC/ING4mt14 tumors. MYC/ING4mt14 tumors had apical lateral tight junction staining within the acini, indicating epithelial polarization of the tumor cells. Expression of ING4mt14 induced tight junction structures in MCF10A breast epithelial cells, but the mechanism by which this occurred is not known. The polarity gene CRB3 could also induce tight junctions in MCF10A cells (28). However, we did not observe increased expression of CRB3 in the cells overexpressing ING4mt14 (data not shown). Currently, it is not clear whether tight junction structures induced by ING4mt14 are directly related to the establishment of cell polarity.
From our results, we infer that a normal function of wild-type ING4 involves tight junction regulation. Hence, disabled ING4 would result in aberrant tight junction formation, which may contribute to tumorigenesis. How might the establishment of tight junctions enhance tumorigenesis? One possibility would be the provision of anchorage required to avoid anoikis. Consistently, MYC/ING4mt14 tumors were less apoptotic compared with the tumors expressing MYC alone. The resulting improvement in survival could contribute to the higher penetrance and metastasis of the MYC/ING4mt14 tumors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

8. Xie Y, Zhang H, Sheng W, Xiang J, Ye Z, Yang J. Adenovirus-avoid anoikis. Consistently, the possibility would be the provision of anchorage required to avoid anoikis. How might the establishment of tight junctions enhance tumorigenesis? One possibility would be the provision of anchorage required to avoid anoikis. Consistently, MYC/ING4mt14 tumors were less apoptotic compared with the tumors expressing MYC alone. The resulting improvement in survival could contribute to the higher penetrance and metastasis of the MYC/ING4mt14 tumors.

Acknowledgments

We thank the past and current members of the Bishop laboratory for helpful discussions, Luda Urisman for her technical assistance in mouse care, and Dr. Kirk Jones (University of California, San Francisco) for consultation with tumor histology.

Grant Support

National Cancer Institute 5K01CA115681 Howard Temim Award (S. Kim), Susan G. Komen Breast Cancer Foundation grant PDF0201190 (A.L. Welm), National Cancer Institute grant 5R35CA044338 (J.M. Bishop), and the G.W. Hooper Research Foundation (J.M. Bishop).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received 02/03/2010; revised 03/26/2010; accepted 04/21/2010; published OnlineFirst 05/25/2010.

Kim et al.

Cancer Research

5162 Cancer Res; 70(12) June 15, 2010

Cancer Research

Published OnlineFirst May 25, 2010; DOI: 10.1158/0008-5472.CAN-10-0425

Downloaded from cancerres.aacrjournals.org on April 20, 2017. © 2010 American Association for Cancer Research.
A Dominant Mutant Allele of the ING4 Tumor Suppressor Found in Human Cancer Cells Exacerbates MYC-Initiated Mouse Mammary Tumorigenesis

Suwon Kim, Alana L. Welm and J. Michael Bishop

Cancer Res 2010;70:5155-5162. Published OnlineFirst May 25, 2010.