The 14-3-3σ Tumor Suppressor Has Multiple Functions in ErbB2-Induced Breast Cancer

Nancy E. Hynes and Tatiana Smirnova

Summary: Ling and colleagues demonstrated that loss of the conditional 14-3-3σ allele results in accelerated HER2/ERBB2-driven mammary tumorigenesis and metastasis. This study underscores the role of 14-3-3σ as a potent tumor suppressor in ERBB2-driven tumor initiation and progression. Cancer Discovery, 2(1); 19–22. © 2012 AACR.

Commentary on Ling et al., p. 68 (5).

Members of the 14-3-3 protein family are present in all eukaryotes, where they have diverse functions as the result of their ability to bind and influence the activity of many cellular proteins. Currently, the list of 14-3-3-interacting proteins is close to 300, and it seems likely that most cellular processes are subject to some type of control by this family. In general, dimers or heterodimers of 14-3-3 proteins recognize specific phospho-serine/phospho-threonine motifs in the proteins to which they bind (RSXpSXP and RXXXpSXp), although nonphosphorylated exceptions have been found. The first studies on 14-3-3 proteins implicated them in control of cell-cycle progression via their ability to bind and inhibit the Cdc25 phosphatases that activate Cdk1. Important roles in cell survival were also uncovered, for example, Akt-dependent phosphorylation of BAD causes it to bind 14-3-3, thereby preventing it from interfering with the pro-survival function of Bcl-XL (reviewed in refs. 1–4).

There are seven 14-3-3 isoforms, identified by Greek letters. The isoform 14-3-3σ, which was studied in the article by Ling and colleagues (5) in this issue of Cancer Discovery, has some unique features that distinguish it from the other 6 members of this protein family. For example, 14-3-3σ is the only isoform that is epithelial cell specific and upregulated by p53 and BRCA1 in response to DNA damage to induce G1-M arrest. Furthermore, in contrast to the other isoforms, expression of 14-3-3σ is often lost in various types of human tumors, pointing to a tumor suppressor role. In tumors, p53 loss can impact 14-3-3σ mRNA levels. It was also found a number of years ago in breast tumors that low 14-3-3σ expression levels can result from aberrant CpG promoter methylation (5).

Low 14-3-3σ levels are found in many types of human tumors due to allelic loss and/or promoter hypermethylation (reviewed in ref. 1); in addition, breast, cervical, ovarian, and bladder cancers have been reported to have low levels of 14-3-3σ, although some tumors, such as ovarian tumors, oral squamous cell carcinomas, and pancreatic ductal adenocarcinomas, among others, show upregulation of the protein (7–9). However, there is a good correlation with hypermethylation and lower 14-3-3σ RNA levels, for example, in the tumors examined by Ferguson and colleagues (6), in which no 14-3-3σ RNA was detected in 45 of 48 tumors. Mhaweh and colleagues (10) also found a close association between 14-3-3σ CpG island methylation and low protein expression levels in patients with breast adenocarcinomas.

However, there is not yet a consensus on expression levels of 14-3-3σ or on its potential prognostic significance in patients with breast cancer. For example, 14-3-3σ expression has been found to be positively and negatively associated with different drug resistance mechanisms in breast cancer cell lines (11, 12). Moreover, in a recent proteomic study, authors detected 14-3-3σ protein in 65 of 68 primary breast tumors examined (refs. 13–18; see Table 1 for more details on breast and other cancers). In summary, the data suggest that 14-3-3σ may only have a tumor suppressor function in a specific subtype of breast cancer.

What can we learn about 14-3-3σ’s role as a tumor suppressor in the article by Ling and colleagues (5) in this issue? First, we need to provide some information on the transgenic models that were used. In the first model, referred to as Erbb2KI, mice carry a Cre-inducible activated Erbb2 allele under transcriptional control of its own promoter and develop focal mammary cancers after an extended period of time. In the second model, referred to as NIC (MMTV-NDL-IRES-Cre), activated Erbb2 and Cre are both driven by the MMTV promoter. Tumors develop more rapidly in this model compared with Erbb2KI transgenic mice. Because the ErbB2KI model has a long period of latency, it is useful for examining chromosomal alterations that might contribute to tumorigenesis, something that was originally examined by Montagna and colleagues (19). In addition to amplification of the Erbb2 locus, consistent deletion or monosomy of chromosome 4 was observed (14). Of note, MMTV-polyoma middle-T-Antigen–induced mammary cancer did not exhibit recurrent loss of chromosome 4, suggesting this genetic alteration is a specific characteristic of ERBB2-driven tumors.

In a follow-up study of 80 tumors, the region on chromosome 4 was refined to a 1.88-Mb area encoding >30 genes (20). Because the distal part of chromosome 4 is orthologous to human chromosome 1p, a region that is often lost in human...
tumors and contains 14-3-3σ, an examination of RNA levels of this isoform was the logical next step. Hodgson and colleagues (20) demonstrated that 13 of 17 tumors had low or no 14-3-3σ expression of another isoform, 14-3-3ζ. Taken together, these studies suggest 14-3-3σ functions as a tumor suppressor in ERBB2-induced mammary cancer.

The article from Ling and colleagues (5) in this issue elegantly confirms this hypothesis by showing that loss of one or both copies of a conditional 14-3-3σ allele leads to accelerated mammary tumor growth in the ErbB2KI transgenic model. The metastatic potential of the tumors was also increased in tumors with 14-3-3σ transgenic model. The metastatic potential of the tumors and contains 14-3-3σ, an examination of RNA levels of this isoform was the logical next step. Hodgson and colleagues (20) demonstrated that 13 of 17 tumors had low or no 14-3-3σ. Taken together, these studies suggest 14-3-3σ functions as a tumor suppressor in ERBB2-induced mammary cancer.

The article from Ling and colleagues (5) in this issue elegantly confirms this hypothesis by showing that loss of one or both copies of a conditional 14-3-3σ allele leads to accelerated mammary tumor growth in the ErbB2KI transgenic model. The metastatic potential of the tumors was also increased in tumors with 14-3-3σ loss. In a heterozygous 14-3-3σ background, the authors show that expression of the remaining 14-3-3σ allele was lost in 8 of 10 tumors and correlated with regions of hypermethylation within the coding region of the gene. They also showed overexpression of Erbb2 RNA in the tumors lacking 14-3-3σ expression and occurring in the absence of gene amplification.

This finding is interesting in light of their previous findings showing that the transcription factor EGR2 and its co-activator CITED1 are upregulated in the ErbB2KI transgenic model and that EGR2 together with CITED1 bind the Erbb2 gene promoter to stimulate its transcription (19). EGR2 also happens to be a 14-3-3σ binding partner; when phosphorylated on serine 376, 14-3-3σ binds EGR2 and sequesters it in the cytoplasm. Following the loss of 14-3-3σ, the transcription factor EGR2 enters the nucleus, where it stimulates Erbb2 transcription (19).

Next, Ling and colleagues (5) concentrated on what loss of 14-3-3σ does to enhance the onset of the ErbB2KI tumors and their increased metastatic potential. To mechanistically study metastasis, they turned to the NIC model, in particular to ex vivo tumor cell lines lacking 14-3-3σ expression that were generated from primary tumors. Reintroduction of 14-3-3σ into these tumor cell lines did not impact proliferation; however, the migratory ability of the cells was impaired, and they had a reduced in vivo metastatic potential. Interestingly, expression of another isoform, 14-3-3ζ, did not affect migration. This finding shows that despite the similarity of proteins in the 14-3-3 family, there are important differences in their target proteins.

The authors previously showed that 14-3-3σ complexes with the polarity protein PAR3 (22). In the absence of 14-3-3σ, PAR3 is expressed, but its membrane localization is lost; introduction of 14-3-3σ restores PAR3 membrane localization and tight junctional and adherens complexes (22). In the new study, Ling and colleagues (5) demonstrate that in tumor cells from the NIC model, loss of 14-3-3σ is also associated with loss of PAR3 from the membrane and loss of adherens junctions.

### Table 1. Existing data on 14-3-3σ expression

<table>
<thead>
<tr>
<th>14-3-3σ Expression in breast and other cancers</th>
<th>Type of analysis</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Downregulated in breast cancer</td>
<td>Promoter hypermethylation: n = 43/50 primary tumors, 32/32 microdissected carcinoma mRNA downregulated in 43/45 primary tumors</td>
<td>6</td>
</tr>
<tr>
<td>Downregulated in breast cancer</td>
<td>IHC: positive staining in 92% of usual ductal hyperplasia and decreased to 23% in invasive ductal carcinoma</td>
<td>14</td>
</tr>
<tr>
<td>Infrequent downregulation in breast cancer</td>
<td>Proteomic analysis and IHC: 65/68 were positive</td>
<td>13</td>
</tr>
<tr>
<td>Downregulated in breast cancer and other types of gynecologic cancers</td>
<td>IHC: 10/43 breast tumors were positive</td>
<td>10</td>
</tr>
<tr>
<td>Downregulated in lung squamous carcinoma metastatic to lymph node</td>
<td>Western blot and IHC: n = 30 paired samples revealed significantly reduced expression in lung metastatic vs. primary tumors</td>
<td>15</td>
</tr>
<tr>
<td>Downregulated by promoter methylation in NPC</td>
<td>Methylation: 63/75 NPC samples Western blot and IHC: downregulation or loss of 14-3-3σ confirmed</td>
<td>16</td>
</tr>
<tr>
<td>Upregulated, correlating with poor prognosis in ovarian tumors</td>
<td>IHC: 8/10 metastatic ovarian tumors and the corresponding primary tumors</td>
<td>7</td>
</tr>
<tr>
<td>Downregulated by hypermethylation in salivary gland adenoid cystic carcinoma</td>
<td>IHC: 1/14 tumor samples positive</td>
<td>17</td>
</tr>
<tr>
<td>Upregulated in PDAC</td>
<td>IHC: 23/33 intraductal papillary-mucinous tumors and 14/14 invasive ductal carcinomas were positive IHC: high is 51/55 in LN metastases, and 49/55 primary tumors were positive</td>
<td>9 18</td>
</tr>
</tbody>
</table>

Abbreviations: IHC, immunohistochemistry; NPC, nasopharyngeal carcinoma; PDAC, pancreatic ductal adenocarcinoma

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Finally, the authors attempted to provide mechanistic insight into the rapid onset of the ErbB2KI tumors that are deficient for 14-3-3σ. First, they showed that these tumors had greater Ki67 staining in comparison with ErbB2KI control tumors. Moreover, an analysis of extracellular signal-regulated kinase (ERK) and AKT (v-akt murine thymoma viral oncogene homolog 1) pathway signaling in 20 tumors, 18 of which had no 14-3-3σ expression, revealed elevated levels of phospho-ERK and phospho-AKT, although only phospho-ERK elevation was significant. In addition, elevated levels of phospho-ERK and phospho-AKT, although ERK levels were slightly elevated. Introduction of 14-3-3σ exhibited diffuse cytoplasmic staining, whereas in 14-3-3σ–deficient cells it was membrane localized and phospho-ERK levels were slightly elevated. Introduction of 14-3-3σ, but also 14-3-3ζ, lowered phospho-ERK levels and caused C-RAF to relocalize from the plasma membrane. Because all 14-3-3 isoforms bind RAF kinases (23), this is perhaps not a surprising result. At this point, it is difficult to conclude that C-RAF is constitutively active in the absence of 14-3-3σ in this tumor model.

Considering that most cells express more than one 14-3-3 isoform and that each can recognize the same consensus phospho-motif, it was not self-evident that the deletion of 14-3-3σ would have such a dramatic phenotype in the ErbB2-induced tumor models. Although not directly shown in this paper, other authors (20) showed that in a panel of 17 tumors, 13 of which had little or no 14-3-3σ expression, all 17 expressed 14-3-3β and 14-3-3ζ. Thus, 14-3-3σ clearly has some specific targets. Indeed, this isoform has a specific role in the final stages of cell division. In its absence, the interphase form of CDK11 is synthesized, not the mitotic form (p110-PITSLRE) required for completion of cytokinesis, which can facilitate the development of pre-cancerous tetraploid lesions (reviewed in ref. 2).

It is important to mention that salivary tumors were also observed in the 14-3-3σ–deficient ErbB2KI tumors. Some human parotid tumors do show elevated levels of ERBB2 as well as 14-3-3σ methylation (17, 24). This finding suggests that ERBB2 overexpression might underlie at least some other cancers in which 14-3-3σ is consistently lost, a possibility that remains to be explored.

We think that the work presented in the article by Ling and colleagues (5) is important for a few reasons. First, because tumors develop slowly and in a sporadic fashion in the ErbB2KI model, in contrast to MMTV-NeuT models, this model is very useful for uncovering other consistent genetic alterations that arise in the tumors; 14-3-3σ loss seems to be a particularly interesting one that will probably provide even more insight into ERBB2 breast cancer once the full spectrum of its binding partners are uncovered. It is also interesting to discuss the transcription factor EGR2, which is free to enter the nucleus after 14-3-3σ downregulation, where it can transcriptionally upregulate ERBB2 (16).

It will also be worth examining whether 14-3-3σ loss and EGR2 upregulation play a role in human breast cancer, particularly in the “ERBB2/HER2-enriched” group, which includes not only tumors with the ERBB2 amplicon but also those only showing elevated ERBB2 RNA (25). Finally, 14-3-3σ also has an important role in regulating cell polarity via PAR3 binding, and in its absence the metastatic phenotype of the ERBB2-induced tumors was accelerated. In a previous report, Ling and colleagues (22) showed that even the adjacent “normal” mammary epithelium of the tumors arising in the ErbB2KI/14-3-3σ-null transgenics showed high methylation of the 14-3-3σ locus. If this really correlates with low levels of 14-3-3σ expression, this finding suggests that downregulation of this isoform could be one of the early changes occurring during tumorigenesis and might influence both cellular polarity and ErbB2 expression to contribute to cancer promotion. In summary, the article by Ling and colleagues (5) reveals the important tumor-suppressive role of 14-3-3σ in ERBB2 breast cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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