

p62: a hub of multiple signaling pathways in HER2-induced mammary tumorigenesis

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We recently reported that depletion of p62 in the background of human epidermal growth factor receptor 2 (HER2) overexpression sensitizes mammary tumor cells to amino acid deprivation, abolishes cellular transformation *in vitro*, and suppresses mammary tumorigenesis *in vivo*. Extensive investigation on the underlying molecular mechanisms has revealed a multifaceted role for p62 in HER2-associated mammary tumorigenesis.

Commentary

In our recent article “Sequestosome 1/p62 facilitates HER2-induced mammary tumorigenesis through multiple signaling pathways”,¹ we reported that the adaptor protein sequestosome 1 (SQSTM1), also known as p62, is required for human epidermal growth factor receptor 2 (HER2)-induced cellular transformation and tumorigenesis in allograft and transgenic mouse mammary tumor models. Our findings are similar to the results of an earlier publication on Ras-induced lung cancer.² These 2 studies, taken together, implicate p62 in potent oncogene-driven epithelial tumorigenesis and suggest that p62 may be a therapeutic target in such malignancies.

p62 is a multidomain protein that governs a diverse array of signaling pathways, and its deregulation has been implicated in Paget’s disease of bone (PDB), neurodegenerative diseases, liver disorders, and cancer. Transcriptional activation of NF-E2-related factor-2 (NRF2) and defective autophagy can both lead to p62 accumulation, whereas p62 in turn regulates NRF2 stability through its interaction with Kelch-like ECH-associated protein 1

(KEAP1).^{3,4} Upon binding to polyubiquitinated substrates, p62 serves as a cargo carrier to proteasomes and the autophagic machinery, where it is degraded along with the cargo.⁵ Furthermore, p62 is involved in nuclear factor kappa B (NF-κB) activation, receptor trafficking, extracellular signal-regulated kinase 1 (ERK1) inhibition, and mammalian target of rapamycin (mTOR) signaling.^{6,7} As a result, disruption of p62 homeostasis impairs disposal of toxic cellular waste, response to oxidative stress, and normal cell signaling.

Our interest in the role of p62 in HER2-induced mammary tumorigenesis was initially triggered by previously published studies that reported increased p62 levels in HER2-positive breast tumors.^{8,9} We found that HER2 overexpression results in p62 accumulation at the protein level, possibly due to HER2-induced autophagy suppression as described in another recent study from our laboratory.¹⁰ Two- and 3-dimensional culture of HER2-expressing *Sqstm1*^{-/-} immortalized mouse mammary epithelial cells (iMMECs) revealed that p62 is required for cell survival upon amino acid starvation and during lumen formation in acinar morphogenesis. As the phosphatase and tensin homolog deleted on chromosome 10 (PTEN)/phosphoinositide-3-kinase (PI3K)/AKT axis is known to promote cell survival and HER2-mediated mammary oncogenesis, we examined the role of p62 in this signaling pathway. Our results demonstrated that HER2-induced activation of Akt, glycogen synthase kinase 3 β (Gsk3β) and β-catenin in mammary cells is dependent on the presence of p62 both *in vitro* and *in vivo*. Furthermore, our studies revealed a newly discovered role for p62 in PTEN regulation. In the presence of p62,

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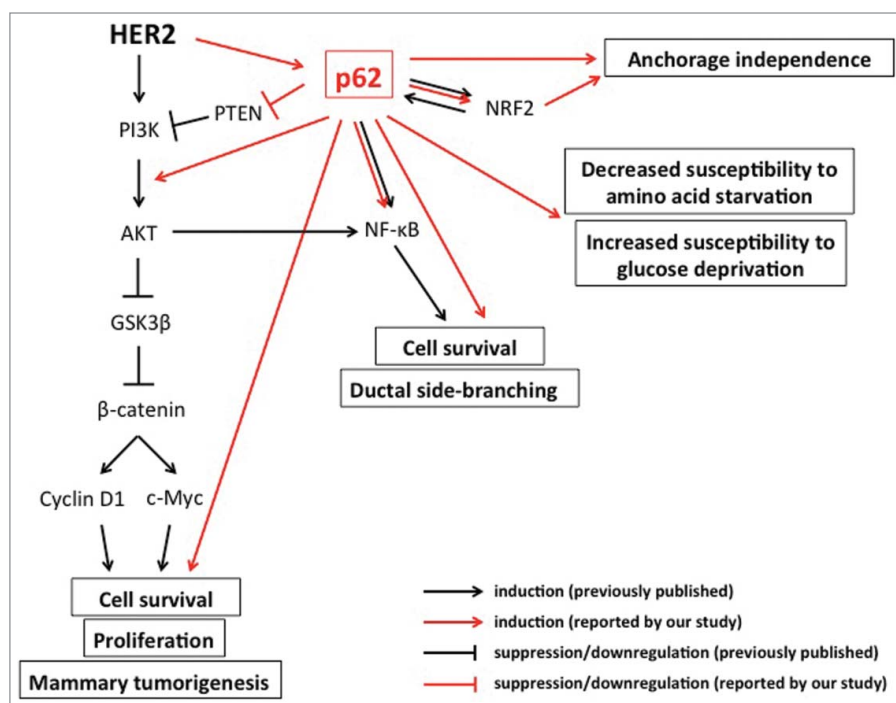


Figure 1. p62 is a hub of multiple signaling pathways in HER2-induced mammary tumorigenesis. In response to HER2 overexpression in mammary epithelial cells, p62 is upregulated at the protein level, which in turn negatively regulates PTEN and activates PI3K signaling, thus leading to tumorigenic signaling cascades. It is also possible that p62 exerts its effects on the AKT/GSK3 β / β -catenin axis independently of PTEN downregulation. Furthermore, p62 activates the prosurvival NF- κ B and NRF2 pathways. As a result, HER2-induced p62 accumulation alters mammary ductal morphogenesis by enhancing side-branching while suppressing lumen formation, and promotes anchorage independence and cellular transformation *in vitro*, as well as mammary tumorigenesis *in vivo*. It is likely that p62 also plays a role in the metabolic reprogramming observed in HER2-positive breast cancer cells, but how this affects mammary tumorigenesis remains to be investigated. GSK3 β , glycogen synthase kinase 3 β ; HER2, human epidermal growth factor receptor 2; NF- κ B, nuclear factor kappa B; NRF2, NF-E2-related factor-2; PI3K, phosphoinositide-3-kinase; PTEN, phosphatase and tensin homolog deleted on chromosome 10.

HER2 overexpression activates the PI3K axis while reducing Pten protein levels *in vitro* and *in vivo*. In contrast, in the absence of p62, HER2-dependent activation of the PI3K pathway is suppressed in association with increased Pten expression, suggesting that p62 is required for Pten downregulation and resultant induction of PI3K signaling in HER2-expressing mouse mammary tumor cells. Whether p62 also activates the Akt/Gsk3 β / β -catenin axis independently of Pten remains to be investigated. Given the established role of p62 in activation of NF- κ B and the NRF2-driven antioxidant response, we also examined the impact of p62 loss on these pathways and found that *in vivo* activation of NF- κ B and NRF2 signaling in response to HER2 overexpression also depends on p62. A limitation of our study is that we did not investigate every pathway

that p62 is known to be involved in, such as the mitogen-activated protein kinase (MAPK) pathway. A role for p62 in HER2-mediated regulation of the MAPK pathway would not be surprising, as p62 has been reported to inhibit ERK1 phosphorylation.⁶ Nevertheless, and in accordance with the complex nature of cancer, our study shows that p62 contributes to HER2-induced mammary tumorigenesis in an elaborate manner that cannot be attributed to a single mechanism (Fig. 1).

Conclusions, Implications, and Unresolved Issues

Our finding that p62 mediates activation of the PI3K/AKT/GSK3 β / β -catenin and NF- κ B pathways in parallel with a

reduction in PTEN levels in response to HER2 overexpression led us to hypothesize that, in addition to potential direct interactions, p62 may also regulate these signaling axes indirectly through its effect on PTEN protein stability. Since p62 participates in proteasome- and autophagy-mediated protein degradation, it is possible that HER2-induced p62 accumulation promotes PTEN degradation by either or both mechanisms. An effect of p62 on *PTEN* mRNA levels cannot be excluded. Thorough exploration of the mechanism(s) involved in p62-mediated PTEN downregulation in response to HER2 activation will certainly be of great interest.

Another finding of our study, which is intriguing and worthy of future investigation, is the observation that while p62 loss sensitizes HER2-expressing mammary tumor cells to amino acid withdrawal, it also renders these cells more resistant to glucose deprivation. Given that autophagy is activated as a prosurvival function in response to amino acid starvation, we entertained the idea that *Sqstm1*^{-/-} HER2-expressing iMMECs cells might be autophagy-defective compared to their wild-type counterparts. However, and in agreement with an earlier report,³ our results showed that neither autophagic flux nor degradation of long-lived proteins is affected by p62 loss. It thus appears that HER2-expressing mammary tumor cells survive in the absence of amino acids via a p62-mediated, but autophagy-independent, mechanism. A possible explanation resides in the hypothesis that p62 may be essential for glycolysis upregulation, which is known to occur in breast cancer cells in response to HER2 activation; as a consequence, amino acid starvation may compromise HER2-expressing mammary tumor cell survival only when p62 is lost and glycolysis cannot be induced to sustain metabolic demands. If this is the case, HER2-expressing mammary tumor cells with intact p62 and metabolism dependent on increased glycolytic rates are expected to be more sensitive to glucose deprivation than their *Sqstm1*-null counterparts, which are probably metabolically adjusted to accommodate defective glycolysis induction under nutritional stress. Since our results are in complete

agreement with this prediction, we propose that p62 plays an essential role in the metabolic reprogramming of mammary tumor cells with active HER2 signaling.

In summary, our work identifies p62 as a hub of signaling and metabolic pathways in HER2-induced mammary tumorigenesis and sets the stage for future studies focusing on the pathophysiology and the discovery of novel therapeutic targets in HER2-positive breast cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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