

The multi-hit hypothesis in basal-like breast cancer

Paul Dent

Department of Neurosurgery; Massey Cancer Center; Virginia Commonwealth University; Richmond, VA USA

It has been known for many years that for a “normal” un-transformed cell to become immortal and subsequently tumorigenic requires multiple pro-oncogenic changes in the levels of protein expression and function. Genes most commonly associated with the process of oncogenesis include: p53 inactivating mutation; hDM2 overexpression; p16 reduced expression; K-/H-RAS activating mutation; PTEN inactivating mutation/deletion; EGFR activating mutation and overexpression; retinoblastoma inactivating mutation and deletion; Cyclin proteins overexpression; CD95 reduced expression; protective BCL-2 proteins overexpression; to name but just a few of such molecules.¹⁻⁵ That the minimally required specific proteins for oncogenesis are not known for many specific tumor types remains a challenge for the rational design of molecular targeted therapies.

In the manuscript by Pires et al., the role of proteins whose function is known to be altered in basal-like breast cancer cells were investigated in an immortal though not tumorigenic mammary tumor cell line, MCF10A.⁶ MCF10A is particularly useful for the present studies as based on expression profiling studies it expresses markers most often associated with the basal-epithelial phenotype.⁷ The authors chose to examine three well described proteins whose functions are altered in basal breast cancer cells; PTEN deletion; p53 mutant inactive; EGFR (also called ERBB1) mutant active. As individual oncogenes, only deletion of PTEN could stimulate 2-dimensional MCF10A cell growth in the absence of exogenous growth factors; and, the effect of a single PTEN deletion was not replicated when cells were grown

in a 3-dimensional culture in soft agar. Interestingly, expression of H-RAS V12 in MCF10A cells did facilitate 3D culture growth; as the H-RAS V12 point mutants specific for activation of ERK/AKT/RAL were not used in this study it is not known which specific downstream pathways are required for this 3D growth effect.^{8,9} The authors then performed analyses using double and triple mutant MCF10A cells. In 2D culture all double mutants except the EGFR + p53 mutant grew robustly. Similar data were obtained when these cells were grown in 3D culture. Of note was that the triple mutant (p53 + PTEN + EGFR) grew more rapidly and formed more colonies than either of the matched double mutants. This supports the multi-hit hypothesis in the MCF10A line. Nevertheless, the triple modified cells were unable to form tumors in SCID mice after 3 mo, suggesting that still more mutations are required for *in vivo* growth. Whether the H-RAS V12 MCF10A cells used in these studies formed tumors was not reported.

A notable difference in signaling between (PTEN + p53) cells and cells expressing the EGFR was further activation of AKT, though this activation of AKT could not explain why (p53 + PTEN + EGFR) grew more rapidly and formed more colonies. A more significant difference was that (p53 + PTEN + EGFR) cells expressed higher protein levels of EGFR and had higher phospho-EGFR levels. Whether (p53 + PTEN + EGFR) cells also express more paracrine EGFR ligands, e.g., TGF α ; HB-EGF, was not reported. Collectively the data in the Pires et al. study argue that multiple pathways downstream and upstream of RAS are required

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Correspondence to: Paul Dent;
Email: pdent@vcu.edu

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to fully transform the basal-cell like cell line MCF10A.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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References

1. Tang Y, Yacoub A, Hamed HA, Poklepovic A, Tye G, Grant S, Dent P. Sorafenib and HDAC inhibitors synergize to kill CNS tumor cells. *Cancer Biol Ther* 2012; 13:567-74; PMID:22406992; <http://dx.doi.org/10.4161/cbt.19771>
2. Mitchell C, Yacoub A, Hossein H, Martin AP, Bareford MD, Eulitt P, Yang C, Nephew KP, Dent P. Inhibition of MCL-1 in breast cancer cells promotes cell death in vitro and in vivo. *Cancer Biol Ther* 2010; 10:903-17; PMID:20855960; <http://dx.doi.org/10.4161/cbt.10.9.13273>
3. Stec R, Bodnar L, Charkiewicz R, Korniluk J, Rokita M, Smoter M, Ciecchowiec M, Chyczewski L, Nikliński J, Kozłowski W, et al. K-Ras gene mutation status as a prognostic and predictive factor in patients with colorectal cancer undergoing irinotecan- or oxaliplatin-based chemotherapy. *Cancer Biol Ther* 2012; 13:1235-43; PMID:22909976; <http://dx.doi.org/10.4161/cbt.21813>
4. Takata M, Chikumi H, Miyake N, Adachi K, Kanamori Y, Yamasaki A, Igishi T, Burioka N, Nanba E, Shimizu E. Lack of AKT activation in lung cancer cells with EGFR mutation is a novel marker of cetuximab sensitivity. *Cancer Biol Ther* 2012; 13:369-78; PMID:22313637; <http://dx.doi.org/10.4161/cbt.19238>
5. Grugan KD, Vega ME, Wong GS, Diehl JA, Bass AJ, Wong KK, Nakagawa H, Rustgi AK. A common p53 mutation (R175H) activates c-Met receptor tyrosine kinase to enhance tumor cell invasion. [Epub ahead of print]. *Cancer Biol Ther* 2013; 14; PMID:23792586
6. Pires MM, Hopkins BD, Saal LH, Parsons RE. Alterations of EGFR, p53 and PTEN that mimic changes found in basal-like breast cancer promote transformation of human mammary epithelial cells. *Cancer Biol Ther* 2013; 14:246-53; PMID:23291982; <http://dx.doi.org/10.4161/cbt.23297>
7. DiRenzo J, Signoretti S, Nakamura N, Rivera-Gonzalez R, Sellers W, Loda M, Brown M. Growth factor requirements and basal phenotype of an immortalized mammary epithelial cell line. *Cancer Res* 2002; 62:89-98; PMID:11782364
8. Carón RW, Yacoub A, Li M, Zhu X, Mitchell C, Hong Y, Hawkins W, Sasazuki T, Shirasawa S, Kozikowski AP, et al. Activated forms of H-RAS and K-RAS differentially regulate membrane association of PI3K, PDK-1, and AKT and the effect of therapeutic kinase inhibitors on cell survival. *Mol Cancer Ther* 2005; 4:257-70; PMID:15713897
9. Carón RW, Yacoub A, Zhu X, Mitchell C, Han SI, Sasazuki T, Shirasawa S, Hagan MP, Grant S, Dent PH. H-RAS V12-induced radioresistance in HCT116 colon carcinoma cells is heregulin dependent. *Mol Cancer Ther* 2005; 4:243-55; PMID:15713896