The Phosphoinositide-3-kinase Signaling Pathway in Breast Cancer

The class I phosphoinositide-3K pathway is a complex network of molecular messengers with significant implications in the role of oncogenesis. Through phosphorylation and dephosphorylation of phosphoinositol rings at the 3' position, a signaling cascade is propelled yielding a genetic response to external stimuli. Over the past 50 years, extensive research has been conducted to discern the true role of this molecule in cell signaling. In the past two decades, molecular research advances have led to the evolution of this investigative field. The following mini-review will discuss past and present research surrounding this pathway by taking a broader approach to provide a more expansive overview of the importance of this signal family.

The Connection to Cancer

At the molecular level, cells are regulated by intercellular molecular signals. These signals are received at the plasma membrane and transduced through various pathways. One of these pathways, identified by Whitman (with Cornell graduate Cantley) in 1988, is associated with the Phosphoinositide-3-kinase (or the phosphatidylinositol-3 kinase).[29, 32] This particular pathway has been revealed to be pivotal in multiple processes, including the mechanism of insulin on glucose uptake in nephritic cells.[8,26]

The most well studied class of PI3Ks is the class IA kinases, categorized based on the specific phosphoinositide substrates and their upstream signals; these are the ones most associated with oncogenesis.[7,17, 34] The downstream effector molecules in this pathway influence key cell cycle processes such as cell growth, proliferation and apoptosis.[6] When the levels of PI3K proteins within cells are altered, either through mutations in the parent gene PI3KCA or some factor which regulates its expression, PI3K's can malfunction. Numerous cancers have already been identified as being influenced by malfunctioning PI3Ks; these include colorectal, liver, lung, and thyroid cancers. [21, 22, 25] Specifically in breast cancers, PI3KCA mutations are present in about 25% of cases.[4]

PI3K signals, like other molecular messages, influence many other signaling pathways and substrates both upstream and downstream. This signaling component is linked to Akt/PKB, mTOR, and Ras effector cascades, all of which influence cell proliferation in cancer. [3,10] Each of these, in turn, have been individually implicated as major sources of oncogenesis. The PI3K signals are just one part of a much larger network. Consequently, the PI3 Kinases are under current investigation.

The Mechanism

The structure of class I PI3K structure has been known for over 30 years.[6] Their structure is broken down into two main domains: the p83 regulatory subunit with the SH2 binding site and a catalytic subunit.[7, 17] The targets of the kinase is belong to a family of membrane bound phosphoinositide molecules.[7,26] Specifically, class I kinases preferentially phosphorylate $PI(4,5)P_2$ to $PI(3,4,5)P_3$.[7,17,34] These phosphorylated PI's have elevated affinities for secondary messengers and kinases.[7] Through binding with this phosphorylated lipid, kinases such as such as Atk and mTOR are brought in close proximity to signaling molecules.[7] These activate further

signaling via phosphorylation of the secondary messengers.

PI3K kinase activity is regulated by numerous upstream signaling molecules. Small molecule signaling via kinase activity is extremely common. Among the signals, G-protein coupled receptors (GPCR) and receptor tyrosine kinases (RTK's), otherwise known as tyrosine kinase receptors, are common activators of PI3K kinase activity. These membrane bound protein complexes are receptive certain ligands. For class Ia PI3K's, the RTK's are the core activator molecules. Examples of such RTK's include receptors in the ErbB-1 pathway.[28] Protein receptors are critical in oncogenesis due to their use in all growth phases. However, there are multiple mechanisms by which these RTK's can alter the PI3 pathway. For example, in invasive lobular breast cancer, EFGR's are overexpressed in the cancerious cells.[12] This overexpression consequentially leads to a quicker and more efficient activation of PI3K molecules, which activate cell proliferation signals down the cascade.

Conversely, inactivation of PI3's via PTEN proteins is also extremely important in regulation of cell processes. Many cellular signals are brief and require deactivation to maintain proper function. In order to terminate the phosphorylation signals of PI3K's, PTEN molecules catalyze the reverse reaction. Specifically in cancerous breast cells, PTEN proteins downregulate PI3K and suppress tumor growth. [14,2] Their expression in cells is vital for PI3 signal functionality; although, the level (or levels) at which expression is regulated is still being established.

Despite the range of knowledge about structure and function, the specific binding actions and effects of PI3K's at the molecular level are still on the frontier of research. The functions of specific residues within the kinases are still being explored via x-ray crystallography of ligand-substrate complexes, a common biochemical technique used to deduce exact intermolecular interactions.[6]

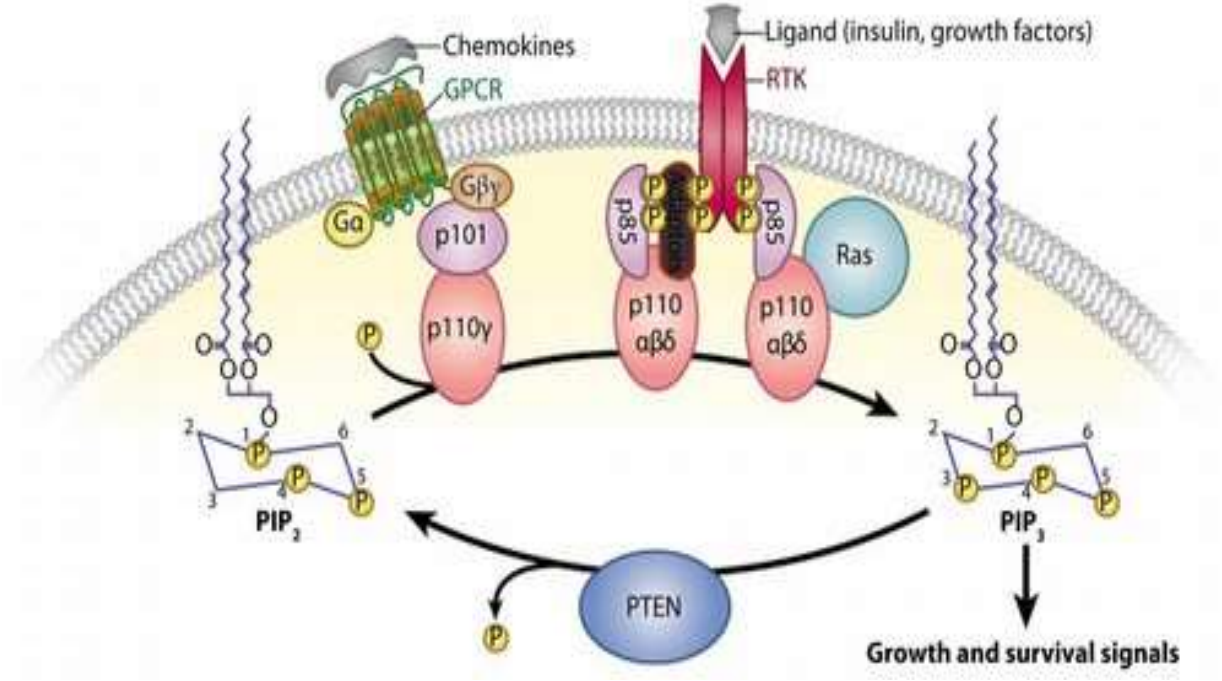

Figure 1: PI3K and PTEN action (11)

Downstream Effectors

The phosphorylated inositol rings embedded in the plasma membrane of cells have the ability to interact with multiple downstream molecules. Although they do not have intrinsic kinase activity, the PI's attract certain secondary messengers such as Akt.[15] Akt's can be activated by other recruited kinases, the most famous being mTOR (mammalian Target of Rapamycin).[15,27] Activated Akt's are significant because of their ability to regulate cell survival through various molecules such as specific caspases. (Cantley, Cardone) The significant effects of PI3K activity on Akt activation have led many scientists to refer to the entire pathway as the PI3K/Akt pathway.

The number of downstream effectors continues to expand as more research is conducted. Other effectors include Ras proteins from the Ras proto-oncogene and MAPK (Mitogen-activated protein kinases). The following image is a good representation of the complexity of the system.

Molecular Cancer Treatment Research

From a molecular standpoint, one source of cancer treatments was seemingly obvious: inhibitors of faulty molecules. Genetic mutations have been shown to be the driving force of oncogenesis. This leads to malfunctioning proteins or changes in expression. In cancer, these changes or malfunctions often change cellular stasis and growth cycles. Inhibition of these specific problematic proteins, either at translation post-transcription or post-translation would help to prevent increased cell proliferation.

Consequently, many techniques have been developed which can target such molecules. Original chemotherapies are based on such drugs and molecular agents. In fact, many individualized techniques such as those describes are still being developed. Recently, a molecule called 4-phenylquinolin-2-yl has been shown to have substantial effects on PI3K's as inhibitory molecules.[1]

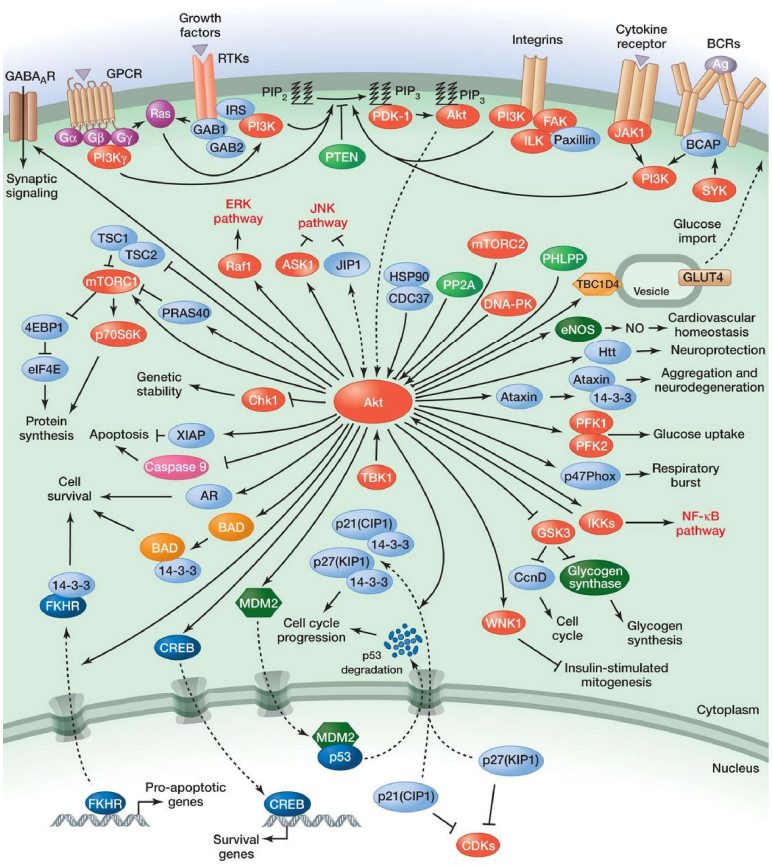

Figure 2- Overview of Signaling Network (18)

Enigmatically, many of these techniques are not effective in the long term. Known as chemoresistance, the mechanisms of

cancer cell resistance to molecular inhibitors have been research extensively in the past two decades. The interconnectivity of signaling molecules and pathways makes compensatory mechanisms in response to chemotherapy commonplace. For example, multiple HER RTK's can respond to similar ligands. Coexistence of numerous HER types in a single cell increase the likelihood of chemoresistance development, suggesting the existence of many options in signal reception.[22] Similar results in other studies support interconnectivity and compensatory mechanisms in order to maintain consistent activation of PI3K and its corresponding downstream signals.[35] Perhaps as an evolutionary mechanism against foreign bodies, the interconnectivity also hinders the anthropogenic efforts to targets single molecules in signal transduction pathways. This has been recapitulated in an more recent study tracking the activity of PI3K during cancer treatment. In response to a chemotherapy known as *trastuzumab*, an inhibitor of the RTK EGFR, PI3K/Akt activity is up-regulated with simultaneous increases in expression of certain RTK's.[2]

Although single molecule inhibition was once a topic of intense research, the focus has shifted towards multi-target treatment plans. By targeting more components of the signal transduction web, it is possible to reduce the ability for cancers to become resistant to single rounds of treatment. Research regarding multi-target therapies either focus on broad effects of single chemicals or a collaborative combination of drugs to yield a similar effect.[35] In a Uterine cancer, combination therapies which target both upregulated HER2 and PI3Ks and show efficacy.[24] In fact, recent studies even suggest that in HER2+ breast cancers dual combination inhibition of PI3K and HER2 proteins is required for therapy efficacy.[38] This is a major area of research in PI3K related cancer therapy.

Basic Science Research and its Link to Cancer

As previously mentioned, specific molecular mechanisms of various enzymes and signaling molecules are poorly understood. For example, under expression of PTEN has been implicated in oncogenesis, but it is not known exactly how this occurs. Recently however, evidence of promoter methylation suggest that under expression of this gene may be at the transcriptional level.[37] By understanding the basic science behind cell signaling within this extremely common signaling pathway, new therapeutic targets are invariably revealed.

One goal for cancer treatment is to discover a specific combination of inhibitions against which evolution is extremely unlikely. In other words, mutations or compensatory mechanisms are not possible or fatal in another way. Although this is unlikely, it is one way in which a cancer may be "cured."

Cancer Identity: The Prognosis

A broad solution for cancer is likely impossible due to the variety of mechanisms by which cancer cells can proliferate. Although certain cancers are caused by PI3K problems and its associated pathways, many cancers are not. In gastric cancers, for example, PI3K's have been shown not to be mutated in correlation with cancerous cells in any statistically significant way.[19] This paper explored the research pertaining to a particular pathway within a certain subset of cancers within the broader topic of breast cancer. The levels of complexity reached and still misunderstood in this one topic alone questions the classification of cancer as one disease.

Although research in PI3K will aid in understanding certain cancers such as breast cancer, other pathways will inevitably need to be researched in order to understand the thousands of types of cancer.

Bibiography

1. Alagumuthu, M. & Arumugam, S. Molecular explorations of substituted 2-(4-phenylquinolin-2-yl) phenols as phosphoinositide 3-kinase inhibitors and anticancer agents. Cancer Chemother. Pharmacol. 79, 389–397 (2017).
2. Alexander, P. B. et al. RTKs in anti-HER2 drug resistance 1 Distinct receptor tyrosine kinase subsets mediate anti-HER2 drug resistance in breast cancer. doi:10.1074/jbc.M116.754960
3. Altomare, D. A. & Testa, J. R. Perturbations of the AKT signaling pathway in human cancer. Oncogene 24, 7455–7464 (2005).
4. Bachman, K. E. et al. The PIK3CA gene is mutated with high frequency in human breast cancers. Cancer Biol. Ther. 3, 772–775 (2004).
5. Bastian, P. Phosphatidylinositol 3-Kinase in the G Protein-Coupled Receptor-Induced Chemokinesis and Chemotaxis of MDA-MB-468 Breast Carcinoma Cells: A Comparison with Leukocytes. Mol. Cancer Res. 4, 411–421 (2006).
6. Batra-Safferling, R., Granzin, J., Mödder, S., Hoffmann, S. & Willbold, D. Structural studies of the phosphatidylinositol 3-kinase (PI3K) SH3 domain in complex with a peptide ligand: role of the anchor residue in ligand binding. Biol. Chem 391, 33–42 (2010).
7. Cantley, L. C. The Phosphoinositide 3-Kinase Pathway. Science (80-.). 296, 1655–1657 (2002).
8. Cantley, L. C. Cancer, metabolism, fructose, artificial sweeteners, and going cold turkey on sugar. BMC Biol. 12, 8 (2014).
9. Cardone, M. H. et al. Regulation of Cell Death Protease Caspase-9 by Phosphorylation. Science (80-.). 282, (1998).
10. Castellano, E. & Downward, J. RAS Interaction with PI3K: More Than Just Another Effector Pathway. Genes Cancer 2, 261–74 (2011).
11. Chalhoub, N. & Baker, S. J. PTEN and the PI3-kinase pathway in cancer. Annu. Rev. Pathol. 4, 127–50 (2009).
12. Ciriello, G. et al. Comprehensive Molecular Portraits of Invasive Lobular Breast Cancer. Cell 163, 506–19 (2015).
13. Depowski, P. L., Rosenthal, S. I. & Ross, J. S. Loss of Expression of the PTEN Gene Protein Product Is Associated with Poor Outcome in Breast Cancer. Mod. Pathol. (2001).
14. The RAS Initiative - National Cancer Institute. Available at: https://www.cancer.gov/research/key-initiatives/ras. (Accessed: 25th April 2017)

15. Franke, T. F., Kaplan, D. R. & Cantley, L. C. PI3K: Downstream AKTion Blocks Apoptosis. Cell 88, 435–437 (1997).

16. Gordus, A. et al. Linear combinations of docking affinities explain quantitative differences in RTK signaling. Mol. Syst. Biol. 5, (2009).

17. Guerrero-Zotano, A., Mayer, I. A. & Arteaga, C. L. PI3K/AKT/mTOR: role in breast cancer progression, drug resistance, and treatment. Cancer Metastasis Rev. 35, 515–524 (2016).

18. Hemmings, B. A. & Restuccia, D. F. PI3K-PKB/Akt pathway. Cold Spring Harb. Perspect. Biol. 4, a011189 (2012).

19. Ito, C. et al. Analysis of PIK3CA mutations and PI3K pathway proteins in advanced gastric cancer. J. Surg. Res. 212, 195–204 (2017).

20. Kalia, M. Biomarkers of Colorectal Cancer. Cent. J. Cancer Biol. J Cancer Biol Res 3, (2015).

21. Karakas, B., Bachman, K. E. & Park, B. H. Mutation of the PIK3CA oncogene in human cancers. Br. J. Cancer 94, 455–459 (2006).

22. Knuefermann, C. et al. HER2/PI-3K/Akt activation leads to a multidrug resistance in human breast adenocarcinoma cells. Oncogene 22, 3205–3212 (2003).

23. Li, J. et al. The PTEN/MMAC1 tumor suppressor induces cell death that is rescued by the AKT/protein kinase B oncogene. Cancer Res. 58, 5667–72 (1998).

24. Lopez, S. et al. Dual HER2/PIK3CA Targeting Overcomes Single-Agent Acquired Resistance in HER2-Amplified Uterine Serous Carcinoma Cell Lines In Vitro and In Vivo. Mol. Cancer Ther. 14, 2519–26 (2015).

25. Pessin, J. E. & Saltiel, A. R. Signaling pathways in insulin action: molecular targets of insulin resistance. J. Clin. Invest. 106, 165–9 (2000).

26. Porta, C., Paglino, C. & Mosca, A. Targeting PI3K/Akt/mTOR Signaling in Cancer. Front. Oncol. 4, 64 (2014).

27. Sarbassov, D. D., Guertin, D. A., Ali, S. M. & Sabatini, D. M. Phosphorylation and Regulation of Akt/PKB by the Rictor-mTOR Complex. Science (80-.). 307, (2005).

28. Sasaki, T., Hiroki, K. & Yamashita, Y. The role of epidermal growth factor receptor in cancer metastasis and microenvironment. Biomed Res. Int. 2013, 546318 (2013).

29. Serunian, L. A. et al. Polyphosphoinositides produced by phosphatidylinositol 3- kinase are poor substrates for phospholipases C from rat liver and bovine brain. J. Biol. Chem. 264, 17809–15 (1989).

30. Stratikopoulos, E. E. & Parsons, R. E. Molecular Pathways: Targeting the PI3K Pathway in Cancer--BET Inhibitors to the Rescue. Clin. Cancer Res. 22, 2605–2610 (2016).

31. Weng, L. P. et al. PTEN suppresses breast cancer cell growth by phosphatase activity-dependent G1 arrest followed by cell death. Cancer Res. 59, 5808–14 (1999).

32. Whitman, M., Downes, C. P., Keeler, M., Keller, T. & Cantley, L. Type I phosphatidylinositol kinase makes a novel inositol phospholipid, phosphatidylinositol-

3-phosphate. Nature 332, 644–646 (1988).

33. Worthington, J., Spain, G., Timms, J. & Timms, J. F. Effects of ErbB2 overexpression on the proteome and ErbB ligand-specific phosphosignalling in mammary luminal epithelial cells. MCP Pap. Press. Publ. (2017).

34. Wymann, M. P. & Pirola, L. Structure and function of phosphoinositide 3-kinases. Biochim. Biophys. Acta - Mol. Cell Biol. Lipids 1436, 127–150 (1998).

35. Xu, A. M. & Huang, P. H. Receptor Tyrosine Kinase Coactivation Networks in Cancer. Cancer Res. 70, 3857 LP-3860 (2010).

36. Yu, D. & Hung, M.-C. Overexpression of ErbB2 in cancer and ErbB2-targeting strategies. Oncogene 19, 6115–6121 (2000).

37. Zhang, H.-Y., Liang, F., Jia, Z.-L., Song, S.-T. & Jiang, Z.-F. PTEN mutation, methylation and expression in breast cancer patients. Oncol. Lett. 6, 161– 168 (2013).

38. Rexer, B. N., Chanthaphaychith, S., Dahlman, K. B. & Arteaga, C. L. Direct inhibition of PI3K in combination with dual HER2 inhibitors is required for optimal antitumor activity in HER2+ breast cancer cells. Breast Cancer Res. 16, R9 (2014).