Genetic Vulnerability: PIK3CA Gene Dependency in HER2-Positive Breast Ductal Carcinoma

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Abstract

In the medical world, a multitude of revolutionary technologies have been developed to improve the process of diagnosing and treating a patient. In the modern era, one of the most innovative developments is the use of CRISPR-Cas9, an enzyme that uses palindromic sequences of nucleotides to bind to and delete or edit DNA. Although heavily studied as a genetic engineering tool, this fascinating protein can also identify genetic targets to improve cancer therapy through CRISPR screening.

Introduction

In order to determine the genetic vulnerabilities in cancer, CRISPR screening can be done. CRISPR(clustered regularly interspaced palindromic repeats) screening is a technique that identifies unique vulnerabilities in cancer cell growth and viability in an unbiased way. Typically, the technique is used to identify which gene, out of thousands of genes, is most responsible for the physiological response being studied by the researchers. Upon the initiation of the screen, the enzyme will locate the target DNA using the guide RNA, bind to the DNA strand, and make a cut that disrupts the DNA of the gene of interest. The CRISPR screen is then carried out for 2-3 weeks in which genes that are enriched or depleted will be identified by comparing them to the initial starting point. For example, genes that are depleted very early on are required for cancer cell growth. Genes that are enriched confer resistance and amplify cell growth.

Background

In past years, these screens have been used to identify genes causing a resistance to specific drugs and those associated with mitochondrial metabolism as referenced in DepMap. For example, in a study conducted by researchers from the Centre of Infectious Disease Research at the Indian Institute of Science, CRISPR screens were used to identify gene dependencies for flaviviruses, responsible for intracellular parasites(S, n.d.).

In an example of breast ductal carcinoma, scientists have performed CRISPR screens on cell lines to identify and locate which mutated genes produce detrimental effects that result in the cancer. To conduct a CRISPR screen of Breast Ductal Carcinoma, Breast cancer cell lines have been established previously such as EFM192A and SUM185PE.

A subset of these cell lines that have been collected can be characterized by the growth receptors expressed. For example, in breast cancer, these are human epidermal growth factor receptor 2(HER2+), estrogen(ER+), and progesterone(PR+). CRISPR screens allow for identification of new vulnerabilities in specific subsets of cell lines. My research looked at genetic vulnerabilities in HER2+ Breast Ductal Carcinoma.

In order to analyze the genetic vulnerabilities of HER2+ Breast Ductal Carcinoma, I used the platform--DepMap Portal(depmap.org). DepMap Portal, a cancer dependency map, fosters new research regarding cancer dependencies by providing

open and free access to intricate visualization and analytical tools of these cancer dependencies. A cancer dependency is essentially a gene that is required for rapid cancer cell growth.

The mutations, genes, and signaling pathways that result in this abnormal growth of cancer cells are heterogeneous and difficult to dissect mechanistically. There are often many genes involved and it is very difficult to comprehend the relationship between these mutations, what they result in, and how to reverse their effects. This cancer dependency map helps researchers not only identify these targets, but also develop a more complex understanding of the dependencies. Through DepMap, scientists have access to a multitude of cancer cell lines that have been previously analyzed by CRISPR screening and other unbiased approaches. The information regarding these cell lines is readily available for scientists to use. The data collected by these cell lines are often used by scientists to develop the most effective therapy for cancer patients.

Method

For the purpose of this paper, Depmap was used to identify what genes play a role in the cell lines of Breast Ductal Carcinoma. The DepMap search tool allows users to search for any cell line in the database through a specific gene, cell line, compound, or lineage. I searched “Breast Ductal Carcinoma” and an extensive list of cell lines were sorted out and generated from the platform's large database. Since I aimed to investigate Her2+ Breast Ductal Carcinoma, I sorted through each cell line and observed if the lineage subtype included the phrase “HER2pos”, translating to HER2+, which is the presence of the HER2 growth receptor found in a subset of breast cancer. After thorough examination of various cell lines affected by Breast Ductal Carcinoma, I noticed the PIK3CA gene was a frequent hit on the CRISPR screen along with many other genes, specifically in HER2+ breast cancer.

The PIK3CA gene is responsible for providing the instructions to synthesize the p110α protein, a key subunit in the enzyme phosphatidylinositol 3-kinase, also known as PI3K. The p110α protein has many isoforms including p110β and p110δ proteins, produced by the genes PIK3CB and PIK3CD respectively. Though all isoforms of the p110 protein have prevalence in various cancers, the p110α protein, encoded by the PIK3CA gene, has a larger effect on tumorous cancers.

In the PI3K signaling pathway, this enzyme sets off a cascade of events that ultimately leads to activation of kinases and downstream phosphorylation. Phosphorylation is the addition of oxygen and a phosphate group. This process activates the cascade of signaling pathways that ultimately leads to rapid cell proliferation, a hallmark of cancer(Mukohara, 2015). Mutations in the PIK3CA gene are prevalent in individuals affected by ovarian, lung, breast, brain, and colon cancers(MedlinePlus, n.d.).

According to select cell lines provided in DepMap, the PIK3CA gene was a frequent hit in the CRISPR screen, meaning it played a vital role in the growth of the cancer in the screened cell line. Specifically, a majority of these cell lines that were most impacted were HER2+ breast ductal carcinomas. Upon activation of HER2 receptors,

the PI3K signaling cascade is known to be stimulated, ultimately resulting in rapid cell proliferation. Given this finding, I was interested in investigating HER2+ breast ductal carcinoma's reliance on the PIK3CA gene.

Since many of the observed HER2+ breast cancer cell lines had the PIK3CA gene as a top hit, I hypothesize that PIK3CA is required for the tumorigenic effect of HER2, specifically in HER2+ breast ductal carcinoma. I reasoned that PIK3CA deletion is deleterious within HER2+ breast cancer cells because these cells are more reliant on this pathway for active cell signaling and proliferation. Thus, when the pathway is shut off due to the deletion of PIK3CA, cell proliferation is greatly inhibited as shown in DepMap(Table 1).

Results

Cell Line (Invasive Breast Carcinoma)	Top 10 Preferentially Essential Genes	Presence of PIK3CA Mutation	Presence of PIK3CA as top dependency	Her2+, Her2-, or Her2+/-?
MDAMB361	PPP1R15B FOXA1 YPEL5 ERBB2 SPDEF CASC3 INTS6 PIK3CA HAUS7 CCND1	Yes	Yes	Her2+
UACC893	FOXA1 PPIL1 INTS6 SNAP23 SRSF11 PIK3CA PPP1R15B GMNN GRB2 CCND1	Yes	Yes	Her2+
EFM192A	PIK3CA KIF11 ERBB2 TRA2B NXF1	Yes	Yes	Her2+

	PLK1 XAB2 YME1L1 NEDD1 PUF60			
BT20	NXF1 THG1L KIF11 SPEF2 SPESP1 MRPS9 WDR47 SH3BGRL2 TCTN3 EARS2	Yes	No	Her2-
SUM149PT	POLR2M ATP6V1H TUBA1B PFDN5 GAR1 TYMS SLC25A38 GGA3 SNAP23 POMP	No	No	Her2-
SUM185PE	DDX39B SRF SNF8 TRPS1 PPP1R14B POLR2M UXS1 RBIS INTS4 ATP5F1B	Yes	No	Her2-
MDAMB453	CDK4 FOXA1 CCND1 ERBB2 GATA3	Yes	No	Her2+

	FGFR4 TYMS SCAP FECH SPDEF			
MFM223	PRKRA TBC1D20 PTAR1 RAB18 FOXA1 INTS6 UXS1 GATA3 NAMPT FGFR2	Yes	No	Her2-
CAL148	SARAF STOML3 NEK11 FAM49A KLK3 ZBTB9 EXOC1 RHOB ZDHHC3 ZNF696	Yes	No	Her2-
HS578T	NCAPG2 ARMC7 NUBP1 DNAJC9 DDX59 CIAO1 MCM3 NHLRC2 HNRNPH1 CHMP4B	No	No	Her2-

Discussion

According to data presented in table 1, it is evident that the presence of the PIK3CA gene has a key role in cell proliferation in HER2+ cells. The cell lines in table 1 were all chosen from a random set of cell lines affected by breast ductal carcinoma. The presence of PIK3CA in the top 10 dependencies in the cell line further solidifies the idea

that the gene is a major dependency. Cell growth is dependent on the presence of this gene. In cell lines MDAMB361, UACC893, EFM192A, the PIK3CA gene was one of the top 10 dependencies, meaning when it was knocked out during the CRISPR screen, the proliferation of cells in the cell line was inhibited. Under further investigation, each of these cell lines was also HER2+, providing evidence that the presence of the PIK3CA plays a major role in cell proliferation of HER2+ breast cancer cells. To further supplement the notion that PIK3CA plays a vital role in cell proliferation of HER2+ breast cancer cells, 6 out of 7 of the HER2- cell lines(BT20, SUM149PT, SUM185PE, MDAMB453, MFM223, CAL148, HS578T) did not have PIK3CA as a top dependency, further supporting the notion that PIK3CA dependency is specific to HER2 expression. HER2+ breast ductal carcinoma has a heavy reliance on the PI3K signaling pathway and the PIK3CA gene to undergo cell proliferation. Therefore, targeting the source of the signaling pathway, the HER2 growth receptor, and targeting PIK3CA would have the most detrimental effect on these cancer cells.

There are already FDA approved drugs to target the HER2 receptor. Trastuzumab is an antibody drug used to treat certain types of cancers, including breast cancer and stomach cancer, that overexpress the human epidermal growth factor receptor 2 (HER2) protein. It is present in the brand-name medication Herceptin. This protein is involved in the regulation of cell growth and differentiation, and overexpression of HER2 is associated with increased cell proliferation, invasiveness, and survival. Trastuzumab works by binding to the extracellular domain of HER2, which leads to the inhibition of downstream signaling pathways that promote tumor growth and survival. This can result in the inhibition of cell proliferation, induction of apoptosis, and enhancement of the immune response against cancer cells(National Cancer Institute, 2023).

Though the drug has proven to be effective at treating HER2+ breast cancers, it has its limitations. Like all cancers, the cells of HER2+ breast cancer develop resistance to drugs over time by creating alternate signaling pathways that would not be affected by the inhibitory qualities of Traztuzumab. Therefore, the best approach to treat HER2+ Breast Ductal Carcinoma is to administer doses of the drug Trastuzumab in conjunction with chemotherapy, surgery, or radiotherapy. While chemotherapy is effective at killing cancerous cells and slowing their reproduction, it does not target the source. Rather, targeted therapies like Traztuzumab bind to and inhibit the HER2 growth receptor, which is required to initiate the growth of HER2+ cancer cells.

Despite some limitations, trastuzumab remains a valuable treatment option for HER2-positive breast cancer, offering hope for patients with this aggressive disease. However, DepMap and my exploration of PIK3CA suggest including drugs that target the PIK3CA protein may improve cancer outcomes in combination with Traztuzumab and chemotherapy. One such drug is alpelisib(Center for Drug Evaluation and Research, n.d.). Altogether, CRISPR screening is a phenomenal tool to discover cancer dependencies and resources like DepMap will help scientists investigate other such dependencies.

Ongoing research is devoted to developing new therapies and therapeutic combinations that can further enhance outcomes for patients with HER2-positive breast

cancer. HER2-positive breast cancer is characterized by its dependence on the PIK3CA gene, which plays a critical role in promoting tumor growth and survival. The targeted therapy trastuzumab, which binds to HER2, can greatly improve the prognosis for patients with HER2-positive breast cancer. Used in combination with chemotherapy, trastuzumab has the potential to enhance progression-free survival and overall survival rates in patients with both early and advanced HER2-positive breast cancer.

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