Tracing major oncogenes of Pancreatic Cancer using Kegg Pathway

Background:

In this manual we are going to explore how to investigate the signaling pathway of some major oncogenes of Pancreatic cancer. A signaling pathway is a series of chemical reactions down a molecular gradient that controls cellular responses. Moreover, they allow us to decipher what the function of genes are and how they affect each other. When an error occurs in a pathway, improper cellular responses can lead to disease proliferation, such as cancer. Understanding cancer pathways can help researchers better treat the disease in a more targeted in a specific way through tools, such as gene therapy. The goal of this manual is to provide sufficient instruction to navigate Kegg Pathway and locate desired genetic pathways for disease research, specifically cancer.

Reference: https://www.cancer.gov/publications/dictionaries/cancer-terms/def/signaling-pathway

Bioinformatic Approach:
Kegg (Kyoto Encyclopedia of Genes and Genomes**)** Pathway is a database containing manually drawn signaling pathways, metabolic pathways, and cellular processes. We will be examining a specific pathway map of Pancreatic cancer and focusing on core oncogenes associated with cancer proliferation. The visualization of the data in a comprehensive map allows easy identification of genetic pathways and how they affect cellular processes and systems. However, manually drawn maps have limitations as there may be pathways unknown. Moreover, if you are looking for specific molecular interactions, there is a chance the database itself does not contain the desired data. There are other softwares and websites to look at molecular pathways, however Kegg Pathway is seemingly the most comprehensive and detailed, therefore containing the most data to reference[1].

Link to Kegg Pathway Database:
https://www.genome.jp/kegg/pathway.html

Link to brief Kegg Pathway tutorial on human disease:
https://youtu.be/fog0bTDbsMY

[1] *Kegg Pathway Database*, https://www.genome.jp/kegg/pathway.html.

TRACING MAJOR ONCOGENES OF PANCREATIC CANCER USING KEGG PATHWAY

Dataset:
The four major oncogenes of Pancreatic Cancer I chose to examine for this tutorial were determined using the following review paper:
Yabar, Cinthya S, and Jordan M Winter. "Pancreatic Cancer: A Review." *Gastroenterology clinics of North America* vol. 45,3 (2016): 429-45. doi:10.1016/j.gtc.2016.04.003

- Oncogenes used:
  - KRAS
  - P53
  - CDKN2A
  - SMAD4

This paper was chosen to focus on because it is one of the most recent (2016) reviews on Pancreatic Cancer. Furthermore, after looking through many articles concerning the same topic, the oncogenes discussed are commonly associated with Pancreatic Cancer and cancer proliferation in general.

Yabar & Winter

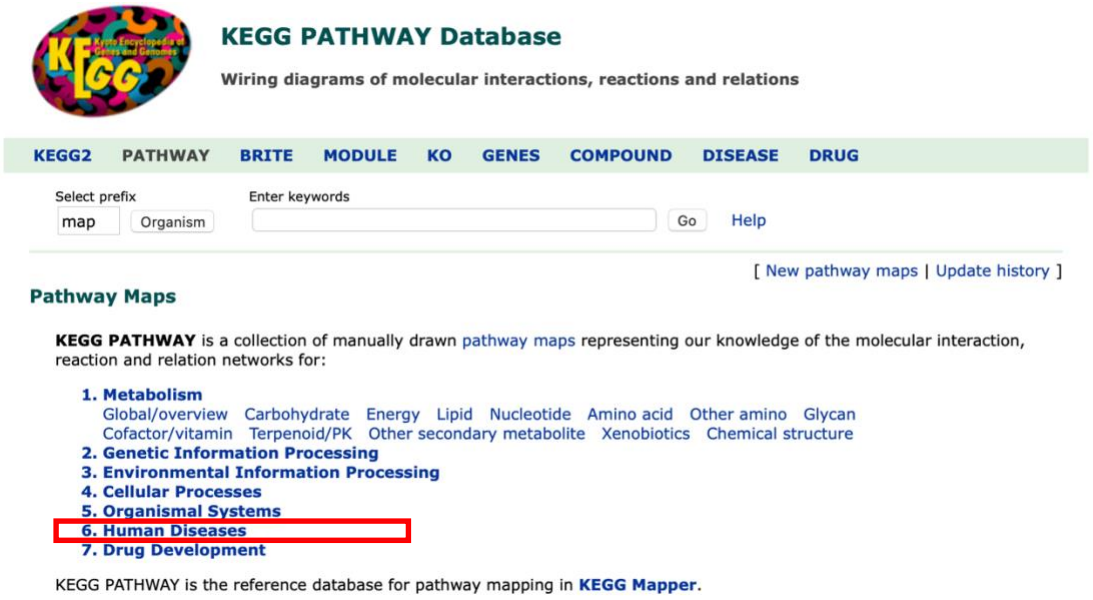
Table 2
Significantly mutated pathways in pancreatic ductal adenocarcinoma

Core Pathway	Gene	Protein Function	Mutation Rate (%)[a]
KRAS signaling	*KRAS*	Oncogene; GTPase; activates MARK activity	100
	MAP2K4	Dual specificity mitogen-activated protein kinase 4; Toll-like receptor signaling pathway	
DNA damage control	*TP53*	Tumor suppressor p53	83
Control of G1/S phase transition	*CDKN2A*	Cyclin-dependent kinase inhibitor 2A; tumor suppressor	83–96
TGF-β signaling	*SMAD4*	Mothers against decapentaplegic homolog 4; BMP signaling pathway	63–100
	TGFBR2	TGF-β receptor type II; regulation of growth	

Abbreviations: BMP, bone morphogenetic protein; TGF, transforming growth factor.
[a] Depending on which gene expressed in sample of tumor studied.
Data from Jones S, Zhang X, Parsons DW, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science 2008;321:1801–6; and Biankin AV, Waddell N, Kassahn KS, et al. Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes. Nature 2012;491:399–405.

Table 2 from Yabar and Winter (2016) displays the genes selected to investigate using the Kegg Pathway database.

FINDING DESIRED DISEASES IN KEGG PATHWAY



There are several categories to choose from when looking for pathway maps. These provide researchers with molecular interactions/reactions for virtually any discovered molecule in organisms. In this case, to trace the major oncogenes chosen (KRAS, P53, CDKN2A, SMAD4) from Pancreatic Cancer, the “Human Diseases” section is the most useful as it contains pathways for over 15 different cancers. This is the section that will be focused on in this manual.

FINDING DESIRED HUMAN DISEASES

Kegg Pathway database already has a map for all the molecular interactions involved in the metastasis of Pancreatic Cancer. Selecting “Pancreatic Cancer” from the list will bring us to the known signaling pathways of the disease.

6. Human Diseases

6.1 Cancer: overview

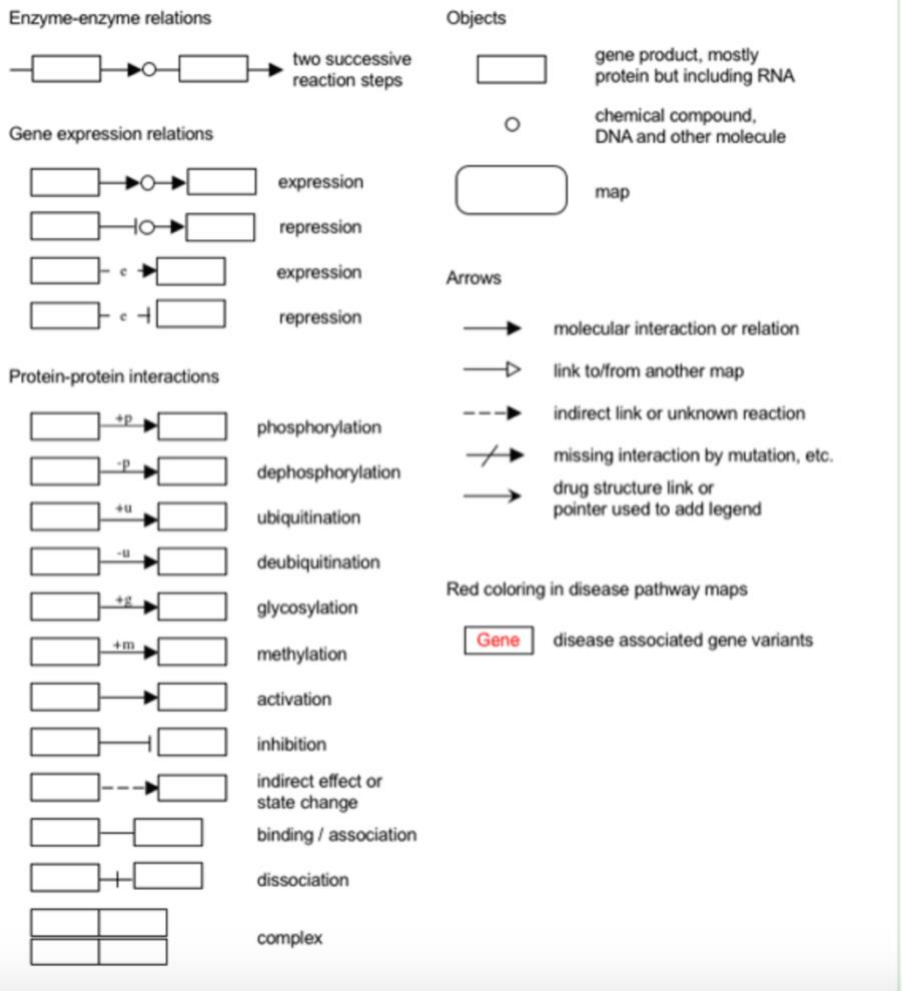
05200 N Pathways in cancer
05202 Transcriptional misregulation in cancer
05206 MicroRNAs in cancer
05205 Proteoglycans in cancer
05204 N Chemical carcinogenesis - DNA adducts *Title changed!*
05207 N Chemical carcinogenesis - receptor activation *New!*
05208 N Chemical carcinogenesis - reactive oxygen species *New!*
05203 Viral carcinogenesis
05230 Central carbon metabolism in cancer
05231 Choline metabolism in cancer
05235 PD-L1 expression and PD-1 checkpoint pathway in cancer

6.2 Cancer: specific types

05210 N Colorectal cancer
05212 N Pancreatic cancer
05225 N Hepatocellular carcinoma
05226 N Gastric cancer
05214 N Glioma
05216 N Thyroid cancer
05221 N Acute myeloid leukemia
05220 N Chronic myeloid leukemia
05217 N Basal cell carcinoma
05218 N Melanoma
05211 N Renal cell carcinoma
05219 N Bladder cancer
05215 N Prostate cancer
05213 N Endometrial cancer
05224 N Breast cancer
05222 N Small cell lung cancer
05223 N Non-small cell lung cancer

READING A KEGG PATHWAY MAP

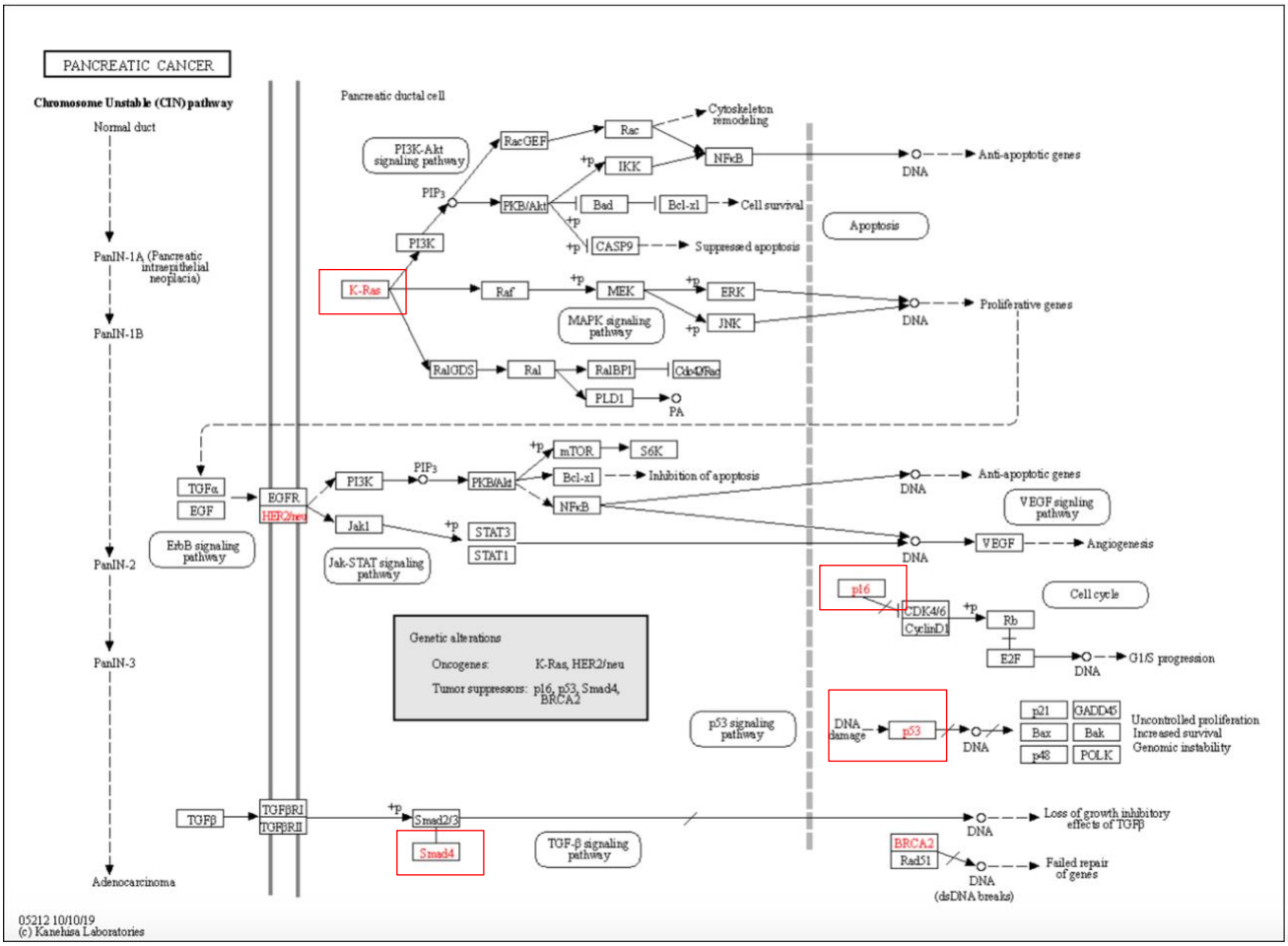
Using this image provided by Kegg Pathway, we can understand how the map is physically organized and understand the molecular reactions/interactions. Many of these interactions will be visualized in the following Pancreatic Cancer signaling pathway map.



Link: https://www.genome.jp/kegg/document/help_pathway.html

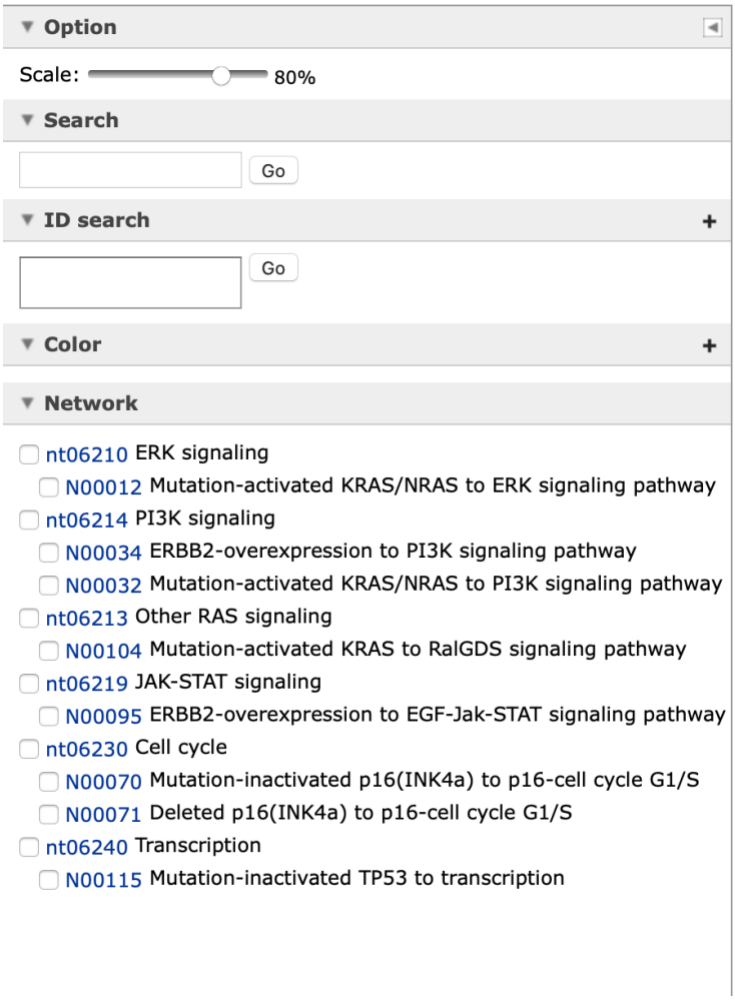
PANCREATIC CANCER PATHWAY MAP

All known genetic pathways have been included in this map of Pancreatic cancer. As shown in the previous image, all genes in red contain genetic alterations/mutations that promote oncogenesis. Our genes of interest have been highlighted in this image with a red box.



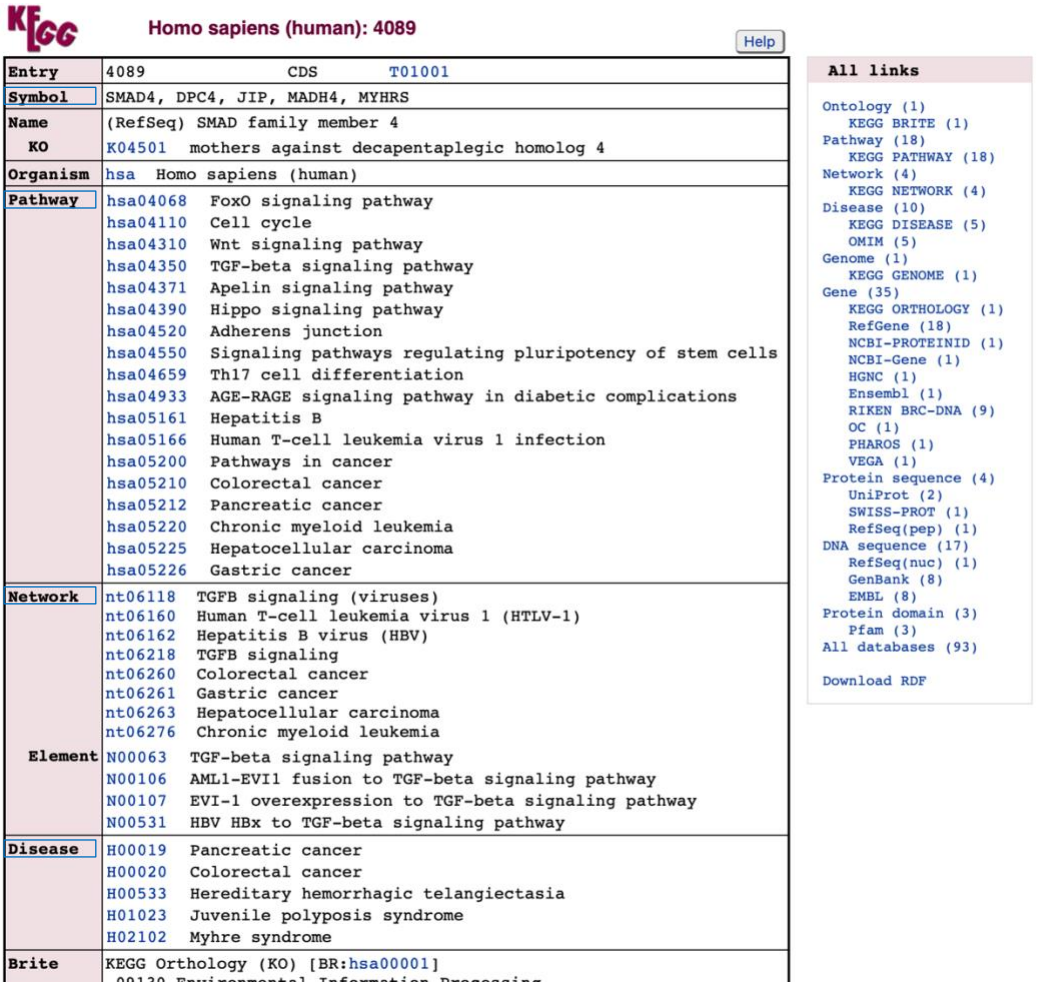
TRACING MAJOR ONCOGENES OF PANCREATIC CANCER USING KEGG PATHWAY

When opening a pathway map, a side panel appears (as shown below). Changing the scale simply changes the size of the map. The search bar allows you to look up any molecule and see if it is in the pathway. The color feature allows to color in desired genes in the pathway, a feature not very useful when just examining a general map. The “Network” drop-down menu displays all the signaling pathways included in Pancreatic Cancer. Mutations to some of these pathways are shown so we can examine what goes wrong for Pancreatic Cancer to occur.



FINDING MORE INFORMATION ON GENES IN MAPS

When reading a KEGG Pathway Map, all genes highlighted by a box can be selected to view more information on the gene itself. When referring to the image below, the "Symbol" column represents another name for the gene, in this case SMAD4. The "Pathway" column displays all other known signaling pathways that your gene is involved in. The "Network" column will take you to another KEGG database, known as KEGG Network. Here, you can find collections of network elements indicating signaling variations, and other interaction/reaction networks in human signaling, disease, and metabolic pathways[2]. Lastly, the "Disease" column, when selected, will take to the KEGG Disease Database to provide more details on the human diseases associated with a specific gene[3].



KEGG

Homo sapiens (human): 4089

Help

Entry	4089 CDS T01001
Symbol	SMAD4, DPC4, JIP, MADH4, MYHRS
Name	(RefSeq) SMAD family member 4
KO	K04501 mothers against decapentaplegic homolog 4
Organism	hsa Homo sapiens (human)
Pathway	hsa04068 FoxO signaling pathway hsa04110 Cell cycle hsa04310 Wnt signaling pathway hsa04350 TGF-beta signaling pathway hsa04371 Apelin signaling pathway hsa04390 Hippo signaling pathway hsa04520 Adherens junction hsa04550 Signaling pathways regulating pluripotency of stem cells hsa04659 Th17 cell differentiation hsa04933 AGE-RAGE signaling pathway in diabetic complications hsa05161 Hepatitis B hsa05166 Human T-cell leukemia virus 1 infection hsa05200 Pathways in cancer hsa05210 Colorectal cancer hsa05212 Pancreatic cancer hsa05220 Chronic myeloid leukemia hsa05225 Hepatocellular carcinoma hsa05226 Gastric cancer
Network	nt06118 TGFB signaling (viruses) nt06160 Human T-cell leukemia virus 1 (HTLV-1) nt06162 Hepatitis B virus (HBV) nt06218 TGFB signaling nt06260 Colorectal cancer nt06261 Gastric cancer nt06263 Hepatocellular carcinoma nt06276 Chronic myeloid leukemia
Element	N00063 TGF-beta signaling pathway N00106 AML1-EVI1 fusion to TGF-beta signaling pathway N00107 EVI-1 overexpression to TGF-beta signaling pathway N00531 HBV HBx to TGF-beta signaling pathway
Disease	H00019 Pancreatic cancer H00020 Colorectal cancer H00533 Hereditary hemorrhagic telangiectasia H01023 Juvenile polyposis syndrome H02102 Myhre syndrome
Brite	KEGG Orthology (KO) [BR:hsa00001]

All links

Ontology (1)
KEGG BRITE (1)
Pathway (18)
KEGG PATHWAY (18)
Network (4)
KEGG NETWORK (4)
Disease (10)
KEGG DISEASE (5)
OMIM (5)
Genome (1)
KEGG GENOME (1)
Gene (35)
KEGG ORTHOLOGY (1)
RefGene (18)
NCBI-PROTEINID (1)
NCBI-Gene (1)
HGNC (1)
Ensembl (1)
RIKEN BRC-DNA (9)
OC (1)
PHAROS (1)
VEGA (1)
Protein sequence (4)
UniProt (2)
SWISS-PROT (1)
RefSeq(pep) (1)
DNA sequence (17)
RefSeq(nuc) (1)
GenBank (8)
EMBL (8)
Protein domain (3)
Pfam (3)
All databases (93)

Download RDF

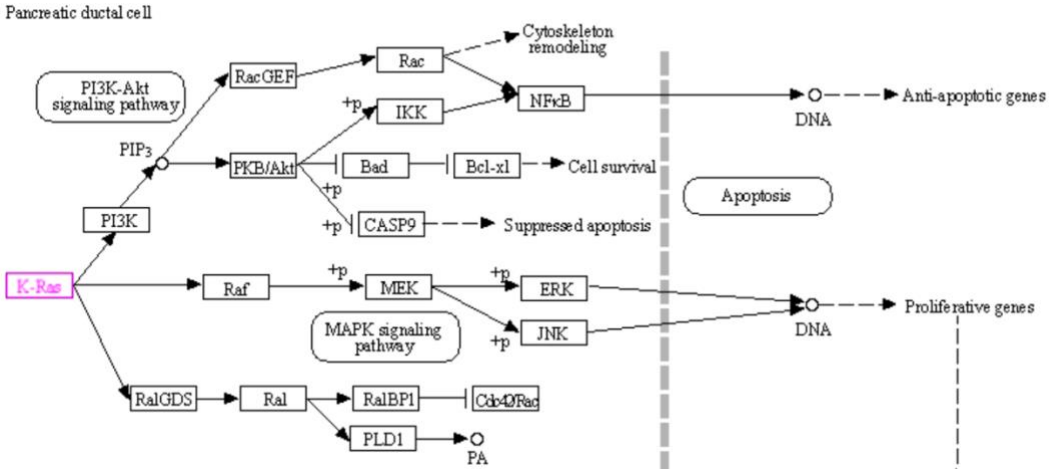
[2] *Kegg Network Database*, https://www.genome.jp/kegg/network.html.
[3] *Kegg Disease Database*, https://www.genome.jp/kegg/disease/.

IDENTIFYING ONCOGENES IN PANCREATIC CANCER PATHWAY MAP

K-RAS GENE

Recent research has demonstrated that K-Ras mutations occur in 25% of all human tumors, making it one of the most commonly activated oncogenes. Studying K-Ras has prompted using this gene as targeted treatment for both colon and lung cancer[4].

The first oncogene being looked at, K-Ras, is shown to mutate in the ductal cell of the Pancreas and affect three different signaling pathways (PI3K, RAF, and RalGDS). More specifically, K-Ras activates these three genes. This is shown through the solid arrow connecting K-Ras to the genes. We can see this genetic mutation produces downstream effects that can lead to the production of anti-apoptotic and proliferative genes, which aids in tumor formation and growth[5].



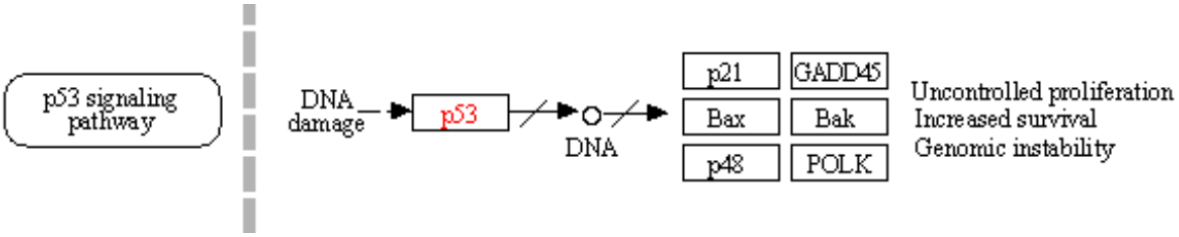
[4] Beganoyic, Sead. "CLINICAL SIGNIFICANCE OF THE KRAS MUTATION." *Bosnian Journal of Basic Medical Sciences* vol. 9,Suppl 1 (2009): S17–S20.

[5] Mustachio, Lisa Maria et al. "Targeting *KRAS* in Cancer: Promising Therapeutic Strategies." *Cancers* vol. 13,6 1204. 10 Mar. 2021, doi:10.3390/cancers13061204

P53 GENE

The p53 gene is a tumor suppressor gene, preventing the growth of tumors. Mutations in p53 have been found in most types of cancerous tumors. Understanding this gene and its molecular network could help in the prevention of tumor formation. However, p53 is just one of many genes commonly found to be associated with cancer growth[6].

When looking at the Pancreatic Cancer pathway map, the p53 gene, when mutated as indicated in the figure, leads to the uncontrolled proliferation of cells, contributing to cancer growth[7]. Furthermore, if DNA damage occurs, p53 becomes mutated, which can cause the expression of at least six downstream molecules. This can result in uncontrolled cell growth, genomic instability, and increased survival of these mutated cells.

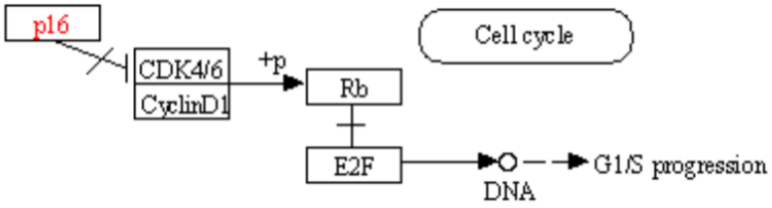


[6] National Center for Biotechnology Information (US). Genes and Disease [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 1998-. The p53 tumor suppressor protein. Available from: https://www.ncbi.nlm.nih.gov/books/NBK22268

[7] Muller, P. A. J., & Vousden, K. H. (2014). Mutant p53 in cancer: New functions and therapeutic opportunities. *Cancer Cell*, *25*(3), 304–317. https://doi.org/10.1016/j.ccr.2014.01.021

CDKN2A GENE

Next, CDKN2A has been found to be frequently mutated or deleted in several different types of cancerous tumors, including melanoma, colorectal and ovarian[8]. It has also been shown to have tumor suppressor gene functions. When looking at the Pancreatic Cancer pathway map, the gene could not be located when initially looking it up using the search bar of the database. However, by clicking on the p16 gene, it is show that CDKNA is another name for p16. When mutated, this gene inhibits the production of CDK4/6 and CyclinD1, as shown by the line with a dash connecting to the two proteins. The pathway shows that mutations of CDKN2A result in errors of the cell cycle, more specially the G1/S phases[9].



Homo sapiens (human): 1029

Help

Entry	1029 CDS T01001
Symbol	CDKN2A, ARF, CDK4I, CDKN2, CMM2, INK4, INK4A, MLM, MTS-1, MTS1, P14, P14ARF, P16, P16-INK4A, P16INK4, P16INK4A, P19, P19ARF, TP16
Name	(RefSeq) cyclin dependent kinase inhibitor 2A
KO	K06621 cyclin-dependent kinase inhibitor 2A
Organism	hsa Homo sapiens (human)
Pathway	hsa01522 Endocrine resistance hsa01524 Platinum drug resistance hsa04110 Cell cycle hsa04115 p53 signaling pathway hsa04218 Cellular senescence hsa04934 Cushing syndrome hsa05163 Human cytomegalovirus infection hsa05166 Human T-cell leukemia virus 1 infection hsa05200 Pathways in cancer hsa05203 Viral carcinogenesis hsa05206 MicroRNAs in cancer hsa05212 Pancreatic cancer hsa05214 Glioma hsa05218 Melanoma hsa05219 Bladder cancer hsa05220 Chronic myeloid leukemia hsa05223 Non-small cell lung cancer hsa05225 Hepatocellular carcinoma

[8] Foulkes, W D et al. "The CDKN2A (p16) gene and human cancer." *Molecular medicine (Cambridge, Mass.)* vol. 3,1 (1997): 5-20.

[9] Agarwal, Payal et al. "Tumor suppressor gene p16/INK4A/CDKN2A-dependent regulation into and out of the cell cycle in a spontaneous canine model of breast cancer." *Journal of cellular biochemistry* vol. 114,6 (2013): 1355-63. doi:10.1002/jcb.24476

SMAD4 GENE

Finally, SMAD4 represents another tumor suppressor gene, suppressing epithelial cell growth through the TGFbeta signaling. Inactivation of SMAD4 occurs frequently in pancreatic and colorectal cancers. Mutations in this gene are not commonly seen through any other cancer types, however, making this mutation very specific to our disease of interest[10].

When inactivated, SMAD4, as shown in the map, becomes an oncogene. If inactivated, SMAD4 can no longer function to suppress tumor growth, affecting the rest of the TGFB signaling pathway. More specifically, this mutation activates DNA production which can result in a loss of growth inhibitory effects provided by these molecules when functioning properly. However, the slashed line through the solid arrow connecting SMAD4 to its downstream effects, shows that there are still missing interactions that need to be determined. It can still be seen that the mutation of SMAD4 can result in unregulated tumor growth. Again, studies have found SMAD4 mutations to be most frequent in pancreatic cancers when compared to other types, which indicates a promising gene for targeted treatment of this cancer[11].

[10] Miyaki, Michiko, and Toshio Kuroki. "Role of Smad4 (DPC4) inactivation in human cancer." Biochemical and biophysical research communications vol. 306,4 (2003): 799-804. doi:10.1016/s0006-291x(03)01066-0

[11] Zhao, Ming et al. "The role of TGF-β/SMAD4 signaling in cancer." *International journal of biological sciences* vol. 14,2 111-123. 12 Jan. 2018, doi:10.7150/ijbs.23230