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# Case Study - Informatics

*Validating PTEN as a Prognostic marker and Therapeutic Target for Multiple Cancers*

## Introduction

Human genome projects and high throughput technologies have given Pharmaceutical Industry vast number of potential Proteins, which has left researchers with the challenge of sifting through enormous data in search of valuable 'Druggable Target' that can cure human disease. Drugs fail in the clinics for two basic reasons: they either don't work or they prove to be unsafe. "Both of these are often the direct result of sloppy early target validation,". Target discovery is the key step in the biomarker and drug discovery pipeline to diagnose and fight human diseases. This requires extensive gathering and filtering of a multitude of available heterogeneous data and information. This is somewhat answered with the help of Proteomics, Disease, Chemical and Clinical databases, but the lack of seamless integration across databases fails to provide holistic picture on Target landscape.

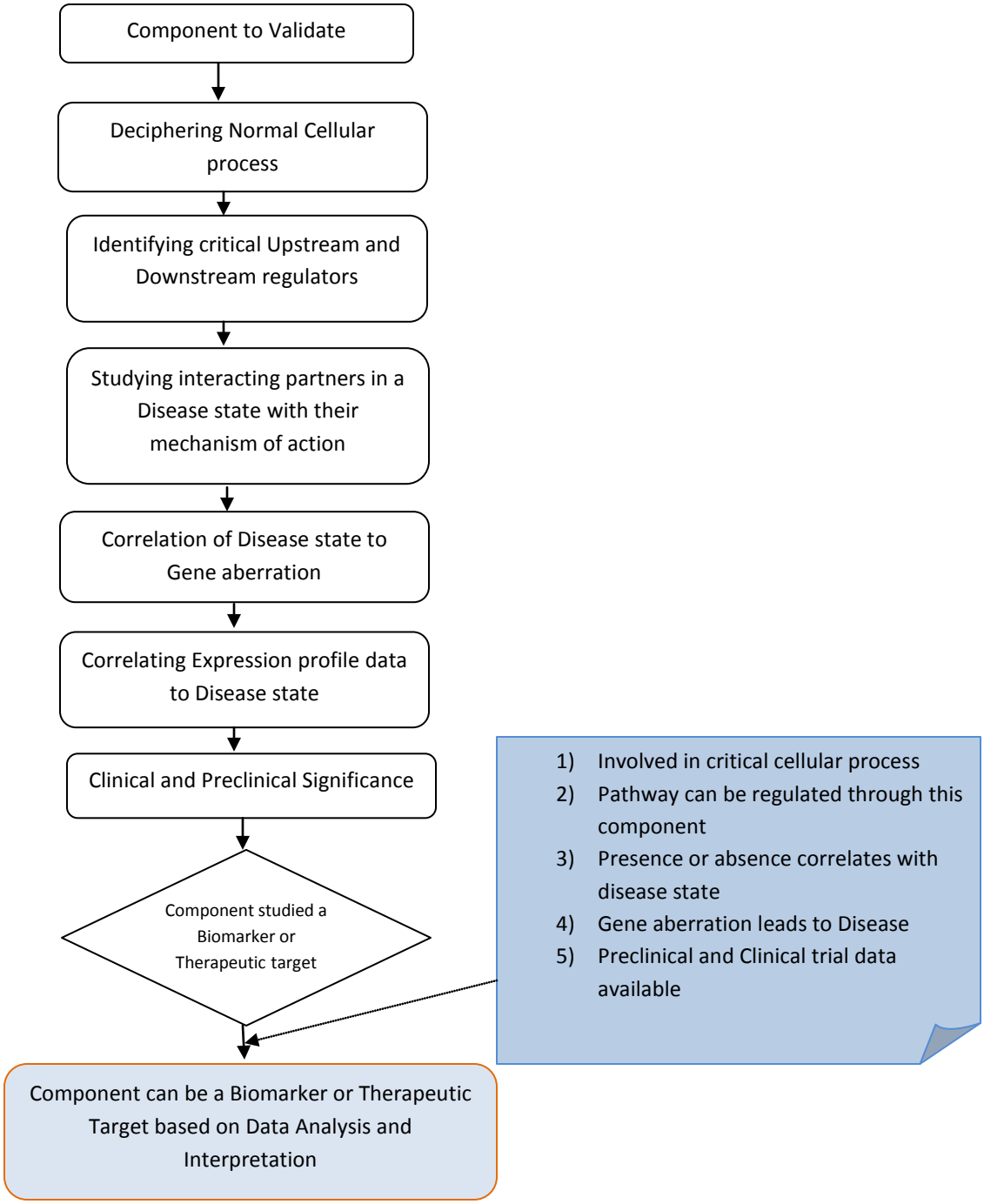
Discovery Informatics Gateway from Jubilant Biosys offers solution to different segments of pharmaceutical industry, ranging from Target Validation to Biomarker Identification and Gene family exploitation. It brings together multiple data types to directly enable faster decision making in drug discovery Process. It has disease, Pathway, Gene aberration and Chemical data points layered into Target and Disease data models.

**PTEN: A Significant player in Cancer Biology**

PTEN (phosphatase and tensin homolog deleted on chromosome ten) a dual lipid and protein phosphatise was first identified as a tumor suppressor gene in 1997. PTEN is a multiple-domain polypeptide of 403 amino acids. It contains an amino-terminal phosphatase domain homologous to chicken tensin and a C2 domain, which mediates the association of signal proteins to plasma membranes (5). The protein level of PTEN is highly correlated with carcinogenesis in a wide range of cancer cases, and hence it is proposed that it should be tightly controlled.

Method Followed:

Process followed by us in carrying out this analysis is represented through a flow chart (given below).



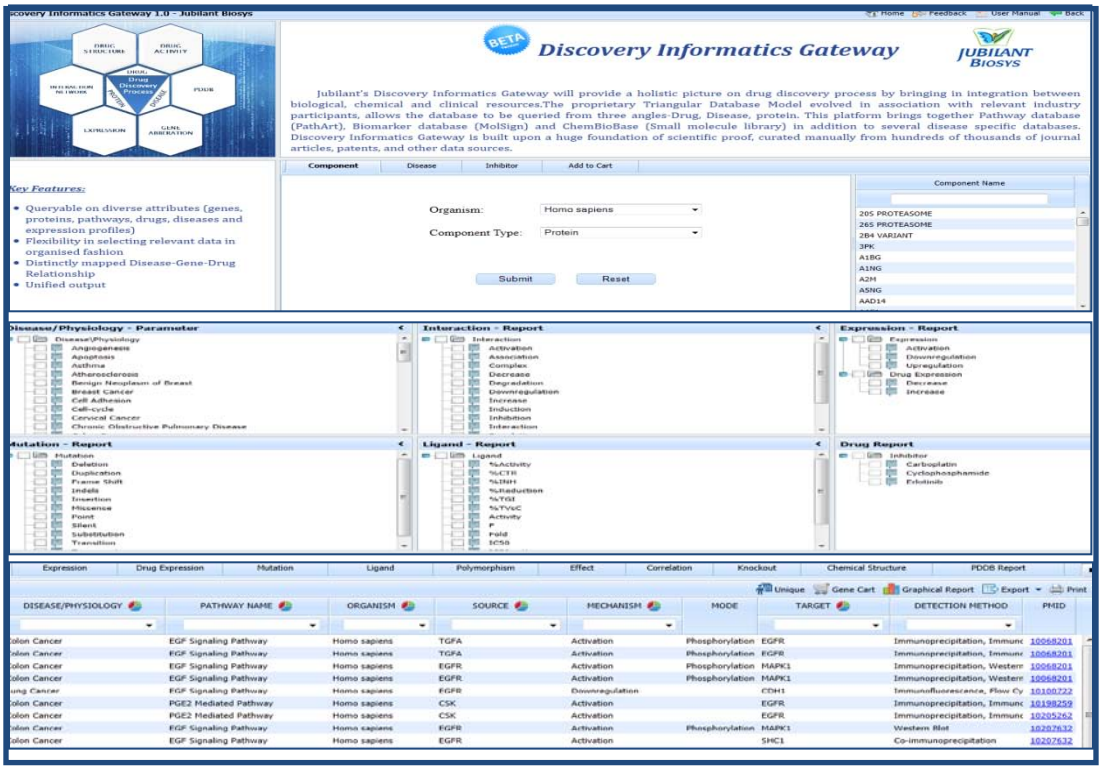



*Fig: 1 Three step option to obtain information on Disease, Expression, Interacting partners and Gene aberration, Clinical and Pre-clinical details for chosen Target*

**Results:**

**What function does PTEN perform in Normal Cellular Process?**

To determine functions performed by PTEN, we filtered information from Interaction based on different physiological processes. We could see PTEN being mapped to four critical processes namely Apoptosis, Cell cycle, Growth and Differentiation and Angiogenesis.

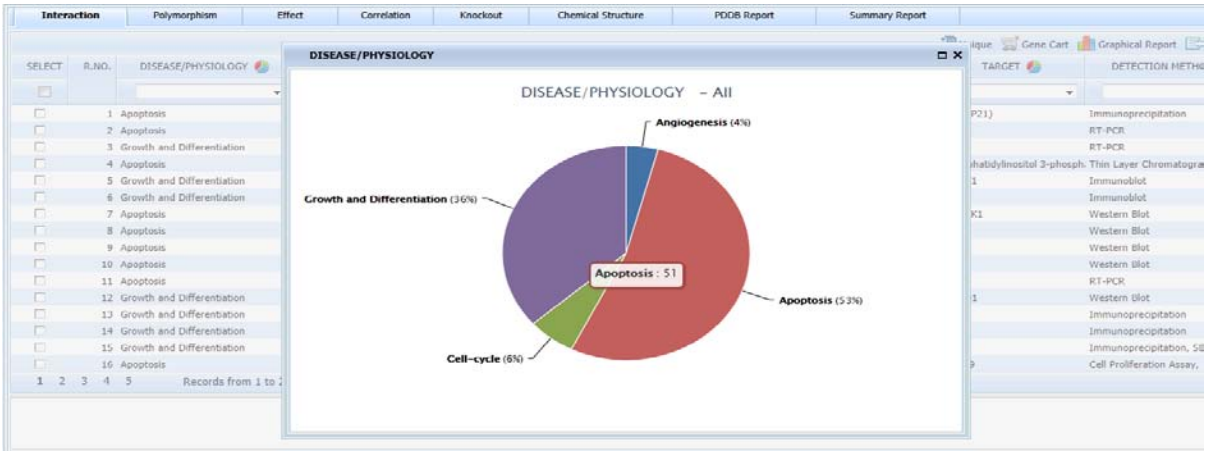



*Fig 2: Representation of PTEN cellular function using a Pie-chart. Based on the function performed, PTEN is classified under four cellular processes namely Apoptosis, Cell cycle, Growth and Differentiation and Angiogenesis*

Next we wanted to identify interacting partners for PTEN - Upstream and Downstream to PTEN. For this we filtered the data from interactions using source (Upstream) and target (Downstream) option.

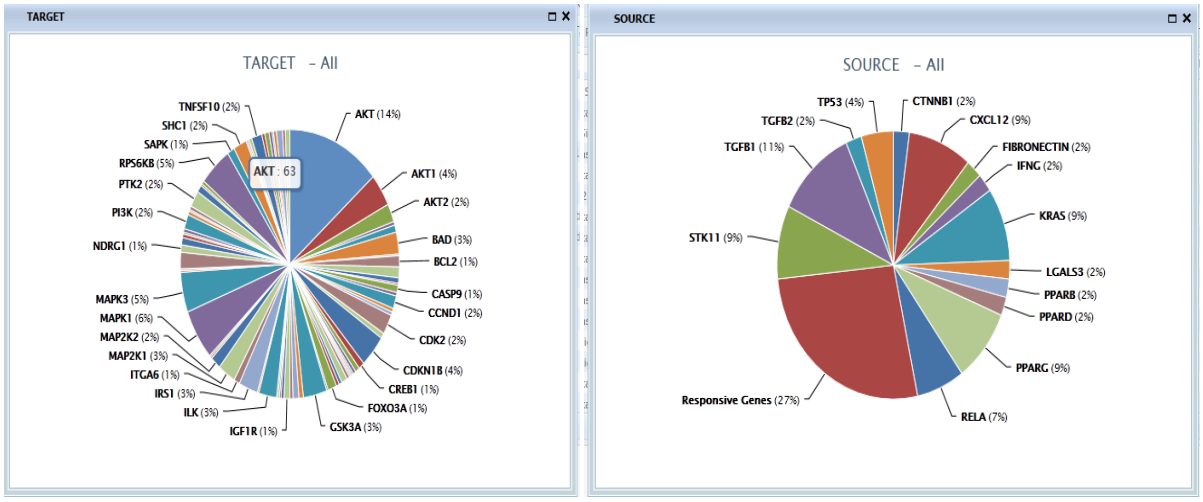



*Fig 3: Components Downstream to PTEN* *Components Upstream to PTEN*

We then exported the results obtained from Pie chart to an excel (Table 1).

| Downstream components | Upstream components |
|---|---|
| IRS1, AKT, MAPK1, MAPK3, SHC1, MAP2K1, MAP2K2, CDK2, ITGA6, NFKB, SAPK, FOXO1A, AKT2, HBEGF, NDRG1, PTK2, ILK, CCND1, TNFSF10, IGF1R, PDK1, FOXO3A, CDKN1B, MYC, CREB1, BCL2, BAD, AKT1, RAS (P21), CCNA, CCNE, CDKN1A, FAK, phosphatidylinositol 3,4,5-triphosphate, phosphatidylinositol 3,4-diphosphate, GSK3A, RPS6KB, AKT3, Calcium, ILK1, AR, PFK, PGK1, PTGS1, HIF1A, PIK3C, VEGF, PI3K, USP4, CTNNB1, GSK3B, CASP9, MAPK (P38), CCND3, FRAP1, BID, MMP2, TIMP2, TP53, CASP3, FASN, TIMP1, BAX, CYCS, PIK3R2, SPP1, F3, CXCR4, IGF1RB, IGFBP4, IGFBP6, CASP7, MAPK, IGF2, ETS2, RAF1, MDM2 | STK11, TGFB1, RELA, PI3K, KRAS, CXCL12, TP53, TGFB2, PPARG, EGFR, IFNG, FRAP1, PPARB, PPARD, FIBRONECTIN, CTNNB1, LGALS3, HDAC |

*Table 1: Downstream and Upstream interacting partners of PTEN*

**PTEN and different Cancers?**

Since PTEN is a part of PI3K-AKT-MTOR pathway and multiple components downstream to PTEN are already well studied druggable targets, we tried to understand the relationship between PTEN and its downstream partners. For this we filtered the data set based on 5 cancers mentioned above and then grouped the results based on mechanism- Increase (Activity or Protein level), Decrease (Activity or Protein level) and Decrease Phosphorylation. Based on our filtration we found ~30 critical downstream components (most of them already worked on Druggable Targets) to PTEN which showed similar mechanism of action across cancers (First time reporting).

| | Breast cancer | Colon cancer | Glioblastoma | Prostate cancer | Stomach cancer |
|---|---|---|---|---|---|
| AKT | | | | | |
| AKT1 | | | | | |
| AKT2 | | | | | |
| AKT3 | | | | | |
| AR | | | | | |
| CCND1 | | | | | |
| CCNE | | | | | |
| CDK2 | | | | | |
| CTNNB1 | | | | | |
| FRAP1 | | | | | |
| PDK1 | | | | | |
| HIF1A | | | | | |
| ILK | | | | | |
| Inositol 3-phosphate | | | | | |
| MYC | | | | | |
| RAF1 | | | | | |
| NFKB | | | | | |
| BAD | | | | | |
| GSK3A | | | | | |
| FAK | | | | | |
| phosphatidylinositol 3,4,5-triphosphate | | | | | |
| CASP3 | | | | | |
| CDKN1A | | | | | |
| CDKN1B | | | | | |
| GSK3B | | | | | |
| TNFSF10 | | | | | |
| TP53 | | | | | |

*Table 2: Components downstream to PTEN along with their mechanism of action across 5 cancers*

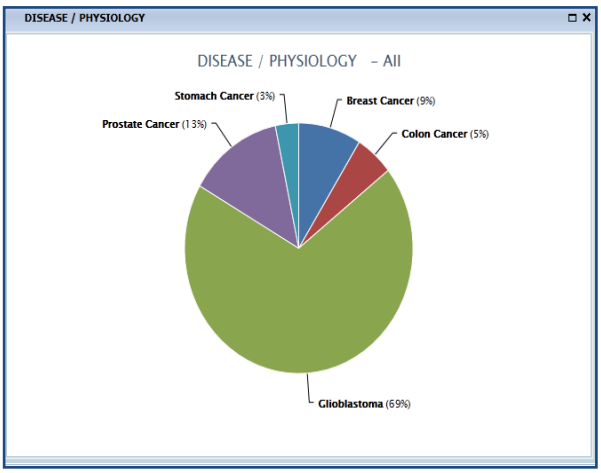
Key for the Table 2:

- Decrease level or activity
- Increase level or activity
- Decrease Phosphorylation
- Data Not Available

**PTEN: Details on Mutation profile across 5 Cancers:**

PTEN is mutated in a wide range of malignancies, especially solid tumors and it is, second only to p53, the most frequently affected tumor suppressor in human carcinomas. Mutations in the gene encoding the tumor suppressor phosphatase and tensin homologue deleted on chromosome 10 (PTEN), leads to uncontrolled activation of the PI3K pathway, which in turn leads to decreased Apoptosis, Increased Cell proliferation and Growth. Hence it is of very much importance to know PTEN mutation distribution across cancers. By looking at the results, for PTEN we had mutation data available for 5 cancers studied (Fig 4).

*Fig 4: Distribution of PTEN mutation across 5 cancers*



We then tried to identify the type of mutation that was prevalent across cancers and for this we opted for graphical view option. The Graph displayed distribution of mutation across cancers based on mutation type. Based on Graphical results we found that Missense mutation was most prominent across cancers followed by Deletion (Fig 5).

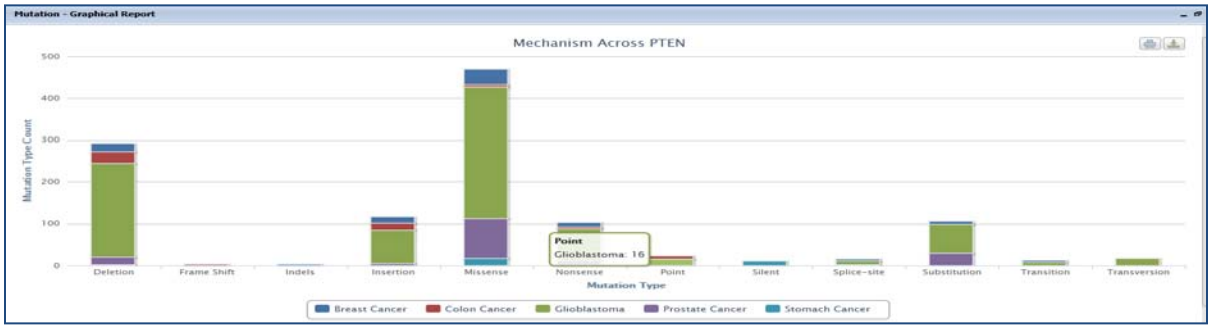
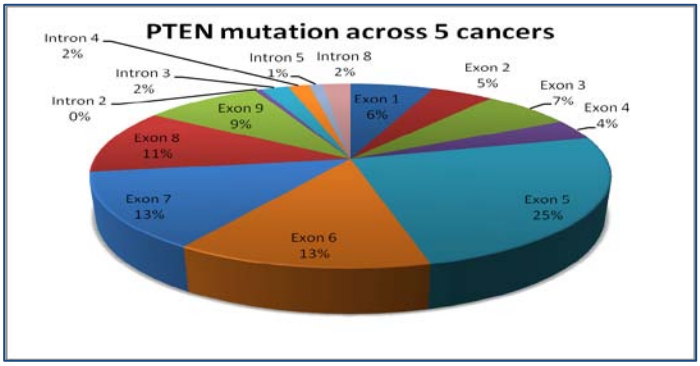



*Fig 5: PTEN mutation distribution across 5 cancers based on mutation type*

We observed that mutation in PTEN was present on all 10 exons and hence we wanted to see how mutation was distributed across Exons. Though mutation was found to be spread across all exons, exon 5 carried most number of mutations (Fig 6).

*Fig 6: Mutation distribution based on Exon for PTEN*




**PTEN and Disease based expression profile**

Our next focus has been to determine the expression profile for PTEN across studied 5 cancers. To perform this analysis we moved from mutation to Expression. Based on the PTEN expression profile results available, we plotted Disease stage VS Percentage of Patient population showing PTEN expression. The most critical observations have been in shown in the graph given below.

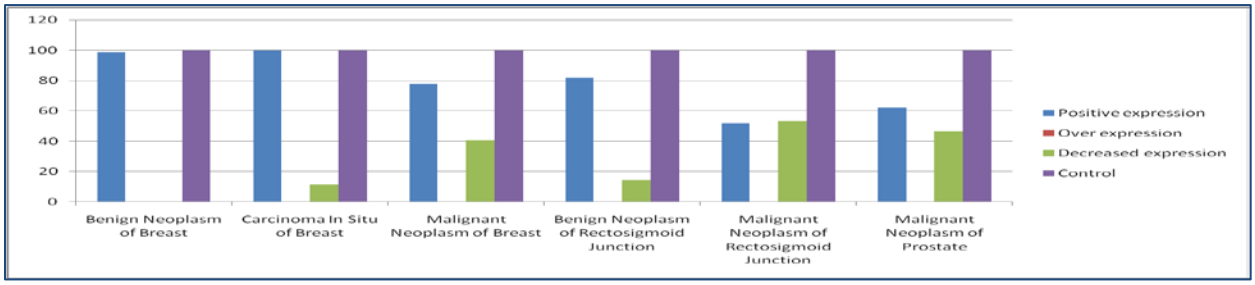



*Fig 7: Disease stage VS Percentage of Patient population showing PTEN expression*

Based on the graphical results we can interpret that PTEN function is seen to be intact in Benign stage of breast cancer, but then as it moves to Carcinoma in situ of breast we can see loss of PTEN and this

percentage of loss increases further in malignant stage. When we compare malignant stage of Breast to malignant stage of Colorectal (Rectosigmoid cancer) to malignant stage of Prostate, we can see that there is significant loss of PTEN happening.

**PTEN and its therapeutic Significance**

PTEN as mentioned above belongs to the most widely studied and therapeutically significant PI3K-AKT pathway. PTEN is a tumor suppressor gene and loss of PTEN leads to cancerous growth- reduced Apoptosis, increased Cell proliferation. Hence PTEN, if it is re introduced into the cell it should be able to impede or reduce cancerous growth. To know about PTEN and studies carried out in Pre clinical stage we have studied more pre clinical information.

| Drug | Combination Drug | Targets acted upon | Experiment model | Effect | PMID |
|---|---|---|---|---|---|
| **EGFR siRNA** | **wild-type PTEN cDNA** | EGFR, PTEN | U251 glioma cells | Downregulation of EGFR expression and Upregulation of PTEN resulted in the suppression of cell proliferation, arrest of cell cycle, reduction in cell invasion and promotion of cell apoptosis in vitro. Glioma transfected nude mice showed reduced tumor growth in presence of combination therapy. | 19728186 |
| **Radiation therapy** | **Adenoviral vector-expressed PTEN** | PTEN | Prostate cancer cells inoculated into the sub cutis of athymic mice | Median tumor size on day 48 was 1030 mm3 in untreated controls, whereas 253 mm3 in mice treated with the combination. AdPTEN strongly inhibits the growth of human prostate tumors when combined with radiation therapy | 16984224 |
| **Radiation therapy** | **GelaTen (microsphere of cationized gelatin hydrogels incorporating PTEN plasmid DNA)** | PTEN | Radiation-resistant PC3-Bcl-2 human prostate cancer cells (PTEN deleted) | PC3-Bcl-2 tumors treated with GelaTen resulted in significant tumor growth suppression compared with control, indicated by the 649 ± 176 mm3 versus 1,252 ± 284 mm3 difference of tumor volume on day 54. | 18644998 |
| **Adenoviral-mediated transfer of PTEN** | | PTEN | Colorectal cancer cells | Treatment of human colorectal tumor xenografts with Ad-PTEN significantly suppressed tumor growth. | 14528320 |
| **Gemcitabine** | **Novel compound-CDF** | PTEN, NFKB, COX2 | Gemcitabine-resistant (MIAPaCa-2) pancreatic cells | CDF suppressed the expression of miR-21, a PTEN target and significantly inhibited tumor growth | 21408027 |

*Table 3: Preclinical studies available using PTEN as a Target*

**Conclusion:**

Through this case study we have made an attempt to understand PTEN from Disease per se and Therapeutic Target per se. Our analysis brings out the upstream and downstream regulators of PTEN and also point towards different cellular processes in which PTEN participates. By identifying and cross comparing downstream regulators across 5 different cancers namely Breast cancer, Colon cancer, prostate cancer, Glioblastoma and Stomach cancer we have for the first time reported 27 different proteins which more or less show similar pattern of expression across cancers. This is a significant finding as most of the downstream regulators like AKT, MTOR, NFKB, TP53, TRAIL etc are already used for cancer treatment and is in different stages of clinical trial. The result obtained from the pre clinical stages point towards the fact that re instating PTEN into cancerous cell can very much reduce or inhibit the cancerous growth. PTEN serves as a good combination therapy partner as in presence of PTEN, AKT is downregulated and this in turn upregulates Apotosis regulators like TRAIL, BAD and induces cell cycle regulators like CDKN1A and CDKN1B. PTEN downregulates AKT dependent and AKT independent tumor regulators like MTOR and RAF. It is also been found that the status of PTEN plays a decisive role deciding the success rate of Receptor Tyrosine Kinase based inhibitors as all these have their downstream signaling passing through PI3K. So PTEN can be used very well as a combination therapy along with these RTK inhibitors. Activation of PPARG through agonists increases functional PTEN protein levels that subsequently induces apoptosis and inhibits cellular growth. Hence treatment of tumor with PPARG agonists like Thiazolidinediones along with other drugs would bring in better treatment results.

PTEN based expression profile clearly indicates towards PTEN's role as a Prognostic marker. As discussed above loss of PTEN is directly correlated to disease stage and with increase in disease progression from benign to malignant there is significant increase in PTEN loss. This makes it possible for us to propose that PTEN can serve as a Prognostic marker, where its loss of function can be correlated to disease progression.

**References:**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