CENTER FOR DRUG DESIGN
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Combinatorial Based Chemistry and High Throughput Screening Methods to Identify Potential Drugs for Pancreatic Cancer

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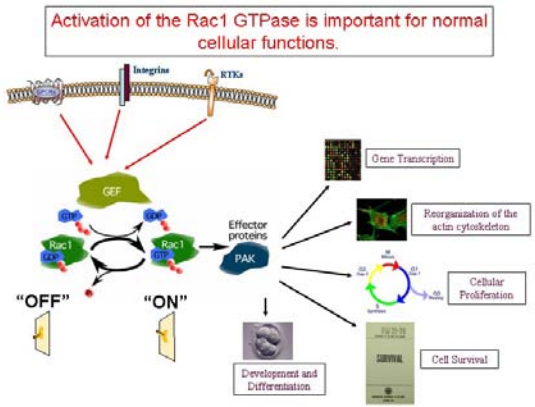
Introduction

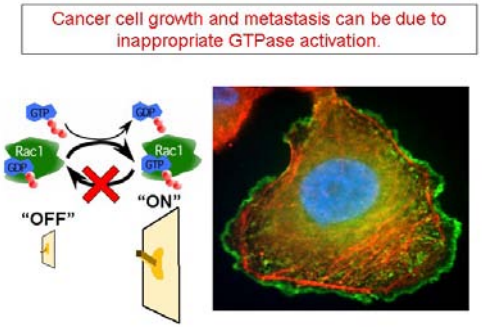
Pancreatic Cancer

- Fourth leading cause of cancer deaths in the US
- Extremely difficult to treat
- Fewer than 3% of patients live 5 years post diagnosis
- We have recently identified that a protein, Rac1 GTPase, is involved in a signaling pathway that leads to pancreatic cancer cell proliferation and survival. Moreover, several studies have identified similar roles for proteins of this class in other human malignancies
- Our hypothesis is that disruption of the signaling pathway will abrogate pancreatic cancer growth and will be less toxic than traditional cancer chemotherapy which attempts to kill cells
- We are therefore developing drug candidates to disrupt this signaling pathway



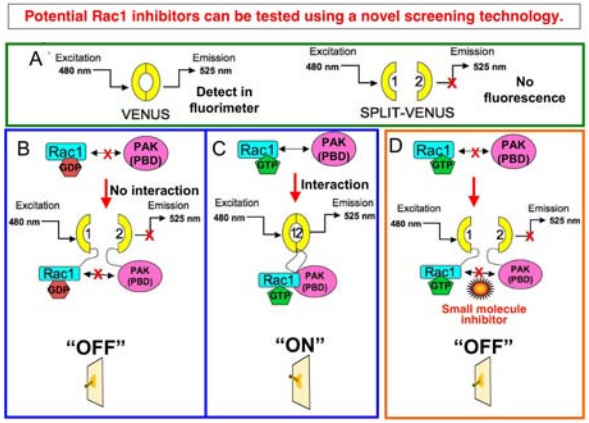
The Rac1 GTPase

- Rac1 is a type of GTPase protein in cells that is critical for carrying-out normal cellular functions
- Rac1 normally binds a small molecule called GDP, essentially keeping the protein in an "off" conformation.
- A variety of signals from outside the cell are transmitted to Rac1 through proteins on the cell surface
- These cell surface proteins interact with Rac1
  - Leading to activation of GEFs (guanine nucleotide exchange factors)
  - The loading of Rac1 with the small molecule GTP.
- This essentially turns Rac1 "on" and leads to multiple cellular consequences through its interaction with other protein partners such as PAK.



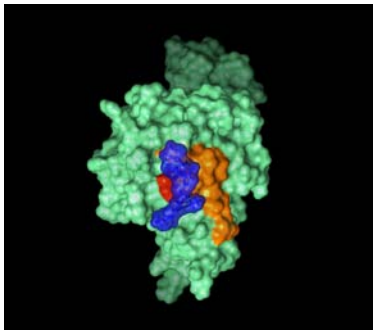
Rac1 and uncontrolled cell growth

Certain types of cancer cells typically are unable to turn "off' Rac1 GTPases, leading to uncontrolled growth and metastasis. The image of the cell on the right (above) displays a morphology consistent with a migratory phenotype and is characterized by an amoeboid appearance. This is typical of a cell in an uncontrolled growth state.



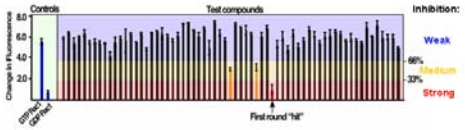
- Panel A (green border), Venus is a fluorescent protein that emits a green light (525 nm), which can be detected using a fluorimeter. The protein can also be split into two halves that are unable to fluoresce independently, but can be brought together to re-establish the fluorescent signal.
- Panel B and C (blue border), the two halves of Venus can be fused to other proteins through molecular biology techniques. In order to screen for Rac1 inhibitors, we have fused Rac1 to the first portion of Venus and PAK the second half of Venus. When Rac1 is in its GDP-bound, "off" conformation, it will not interact with PAK, and there will be no fluorescence (Panel B). When Rac is GTP-bound and is turned on, it will interact strongly with PAK, two halves of Venus generate a fluorescent signal (Panel C).
- Panel D (orange border), Rac1 inhibitors can be identified by the inability of GTP-bound Rac1 to bind PAK and generate a fluorescent signal, indicating Rac1 is essentially being turned off.

Design of Drug Candidates



- We have computationally examined nearly three hundred-thousand small molecules for their ability to disrupt the RAC1 interactions.
- The image above details our model. The protein is colored in green, while important sites for interaction with PAK are shown in orange. Important sites for interaction with other signaling proteins (GEF) are shown in red.
- In blue, a potential drug fits snugly onto the surface of the protein, covering a significant portion of the region associated with PAK and GEF interactions. Such potential drugs are then synthesized in our labs or are purchased from chemical suppliers.

Compounds that have been selected as potential inhibitors of the Rac1/PAK interaction by computer modeling are tested for an effect *in vitro*.



- Using our split Venus screening assay, about 1,000 compounds identified by our computer modeling as potential Pac-Pak modulators were tested for their ability to disrupt the Rac1-PAK interaction *in vitro*
- The graph above shows representative data for 65 of these compounds, with one compound strongly inhibiting the rac1/PAK interaction. **Currently 35 compounds have been identified as strong inhibitors in this assay.**

Conclusion

- **We have developed a novel assay to screen drug candidates for disruption of the Rac-Pak activation**
- **We have developed a large library of drug candidates that may have activity against other important disease targets in addition to cancer**
- **We have discovered 35 drug candidates that have potential to block the Rac1 signaling pathway and therefore inhibit pancreatic cancer cell growth**
- **We are further evaluating these 'hits' for their anti-cancer properties**