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13. ABSTRACT ***(Maximum 200 Words)***

I have proposed to identify peptides that bind to the vasculature of prostate cancers by using a technique developed in our laboratory called "*in vivo* phage display", and then to use these peptides to isolate their vasculature-specific receptors. Specific markers for the vasculature of prostate cancers can be good targets for anti-prostate cancer drugs and will lead to a greater understanding of prostate carcinogenesis. I have also taken an alternative approach to identify prostate cancer vasculature-specific markers by directly comparing the mRNA profiles between endothelial cells from prostate tumors and normal organs.

For this project I have accomplished the following:

¥ Established an expression cloning strategy using phage-displayed RGD4C peptide and αvβ3-expressing HEK cells as a model system.

¥ Established three ecdysone-inducible R-Ras cell lines for testing cDNA microarray and GeneChip technologies.

¥ Used sequential *in vitro* transcription to setup a highly sensitive gene-profile assay that is 10,000 times more sensitive than the original method.

¥ Isolated highly pure endothelial cell populations from various organs and prostate tumors of Tie-2/LacZ tansgenic mice.

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Jih-tung Pai 5/27/00

PI - Signature Date

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Introduction:

I have proposed to identify peptides that bind to the vasculature of prostate cancers by using a technique developed in our laboratory called "*in vivo* phage display". These peptides would be able to home to prostate cancers when injected into the circulation.

I would then use these peptides to isolate their vasculature-specific receptors. These receptors could be specific markers for the vasculature of prostate cancers and lead to a greater understanding of what kinds of vascular surface proteins are up-regulated during prostate carcinogenesis. Both the resulting peptide ligands and receptors would enable us to develop more efficacious but less toxic anti-prostate cancer drugs.

Body:

I started to develop a expression cloning method that used phage-displayed RGD-4C peptide as a probe. The RGD4C peptide tightly binds to $\alpha v\beta 3$ integrin and is a tumor-targeting peptide. I transfected HEK293 cells with either $\alpha v\beta 3$ cDNAs or vector control and determined that phage-displayed RGD-4C peptide bound to the $\alpha v\beta 3$ transfected cells relative to cells transfected with vector alone. I also performed immunohistochemical staining with anti-phage antibody, and observed a distinct staining pattern only with the $\alpha v\beta 3$ transfected group. These data indicates the feasibility of using phage-displayed RGD-4C peptide as a probe to identify $\alpha v\beta 3$-positive cells in a cDNA library expressed in HEK293 cells.

Concurrently, I performed *in vivo* phage display on xenograft tumors in nude mice. The screening process was more difficult and took longer than expected because of the variability in tumor size and composition, along with the time necessary to grow tumors of a proper size. So far, I have not obtained tumor-targeting phage that are suitable as probes in an expression cloning system.

I have initiated an alternative approach to identify receptors that are specifically expressed on the endothelial cells that comprise prostate cancer's vasculature. This approach involves isolating endothelial cells from prostate tumors and comparing their mRNA expression profile with endothelial cells from normal organs. The technology of cDNA microarrays and GeneChips have proven to be very useful in analyzing mRNA expression profiles. The sensitivity of the assays have been improved to a degree that it is feasible to do the assay with a small amount of endothelial cells, and I should be able to recover sufficient material from a few mouse prostate tumors.

This approach has several steps:

1. Establish inducible cell lines as source of mRNA.

To this end, I have established three inducible stable cell lines: pIND-R-Ras-CA, pIND-R-Ras-DN, and pIND-R-Ras-WT. Respectively, these three cell lines express constitutively active R-Ras, dominate-negative R-Ras, and wild type R-Ras under the control of the inducible ecdysone promoter. R-Ras was shown in our laboratory to inccrease cell adhesion. This inducible system is an ideal test for cDNA microarrays and GeneChips because we can directly compare the non-induced and induced states. The nature of the inducible system allows me to use R-Ras up-regulation as an internal positive control. I have identified several up-regulated and down-regulated genes upon induction of constitutively active R-Ras. One up-regulated and one down-regulated gene have been further verified by northern blot analysis.

2. Use sequential *in vitro* transcription to setup a high-sensitivity gene-profile assay.

It is essential to have a high-sensitivity assay for cDNA microarray and GeneChip analyses because one can only get a small amount of high purity endothelial cells from mouse tumors and organs. I have setup this assay in collaboration with Dr. William Wachsman, the director of UCSD's GeneChip facility. We have reduced the required starting material from 100 μg to 10 ng of total RNA while essentially maintaining the same accuracy. This represents a 10,000-fold increase in the assay's sensitivity.

3. Isolate highly pure endothelial cells from various organs and prostate tumors of mice.

It is crucial that we can isolate a highly pure population of endothelial cells in order to get correct and useful expression profiles. I obtained Tie-2/LacZ transgenic mice from Jackson laboratory to make the isolation process and purity verification easier. Tie-2/LacZ transgenic mice express the β-galactosidase gene under the control of Tie-2's promoter. The Tie-2 promoter is only turned on in endothelial cells. I can easily isolate LacZ positive cells by using a fluor-producing substrate in combination with a cell sorting procedure. This procedure has been established for the spleens and lungs of Tie-2/LacZ transgenic mice. I am in the process of breeding Tie-2/LacZ mice with the TRAMP prostate cancer model mice, so I can obtain endothelial cells from prostate tumors.

List of key research accomplishments:

- ¥ Established an expression cloning strategy using phage-displayed RGD4C peptide and αvβ3-expressing HEK cells as a model system.
- ¥ Established three ecdysone-inducible R-Ras cell lines for testing cDNA microarray and GeneChip technologies.
- ¥ Used sequential *in vitro* transcription to setup a highly sensitive gene-profile assay that is 10,000 times more sensitive than the original method.
- ¥ Isolated highly pure endothelial cell populations from various organs and prostate tumors of Tie-2/LacZ tansgenic mice