Report “*Cancer Cell Feature Reversion as a Novel Therapeutic Approach to Cancer*”

Dr. Carol Heckman, PhD

Proposal Summary. Carol Heckman, a professor at Bowling Green State University, has a method for assaying features that develop in cancer cells. Cancer cells lose filopodia. These have a sensory function in neural cells and therefore may play the role of informing cells about the density of neighboring cells. Restoration of the normal phenotype, by reversal of such features, may be an effective strategy for developing new chemotherapy agents. The features characteristic of cancer cells are replicated by activation of one isoform of protein kinase C by the tumor promoter, phorbol 12-myristate 13-acetate. They could be prevented by transcriptional abrogation of PKC-epsilon followed by PKC-epsilon degradation (double knockdown). Dr. Heckman’s hypothesis is that the phosphorylation of several protein species by PKC accounts for the changes in cancer cell phenotype. Fortunately, substrates can be identified by virtue of the fact that they will be phosphorylated in cells exposed to the tumor promoter, whereas cells treated to double knockdown will not have PKC and will contain the native, unphosphorylated substrates.

With a 2008 Seed Grant from NRGF, Dr. Heckman and her colleagues will conduct a pilot study of the sequences that are “tags” of the crucial PKC substrates. Knowledge of these sequences will lead to the identity of the proteins, since nearly all protein sequences are available in publicly supported databases. The knowledge of these sequences is needed to demonstrate that the project is feasible and win funding from the Public Health Service. The unphosphorylated/phosphorylated (UP) peptide pairs indicative of these substrates will be found by isolating different weight classes of proteins, making tryptic digests, and analyzing the resulting peptides by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). The innovative aspect of the project is that it taps the unexploited potential of suppressing the ability of cancer cells to migrate. As a consequence, the project will lead to treatments that increase the therapeutic options available to cancer patients. The basic advances will improve outcomes for patients with breast cancer, by allowing them to elect a therapy with more effective, less toxic agents.

REPORT:

Working with a graduate student, Surya Amarachintha, and Dr. Nicholas Sherman, Director of the Keck Biomedical Mass Spectrometry Laboratory at the University of Virginia, my laboratory has identified some proteins that may be critical substrates of PKC-epsilon. We concentrated on a broad region of the SDS-polyacrylamide gel from 37-50 kDa, where there appeared to be differences between the control and the double knockdown experimental sample. We were looking for a protein that would be phosphorylated in the control sample, but would lack phosphate in the experimental sample where the PKC-epsilon was missing, in which case the substrate could be dephosphorylated by a phosphatase. Instead, the evidence suggested that some proteins were hyperphosphorylated in the experimental sample. This result would have occurred if the phosphorylated state were regulated by a phosphatase which was in turn activated by PKC-epsilon. Inspection of the MALDI results for the 37-50 kDa region showed two

peptides of this type, as illustrated in Fig. 1.

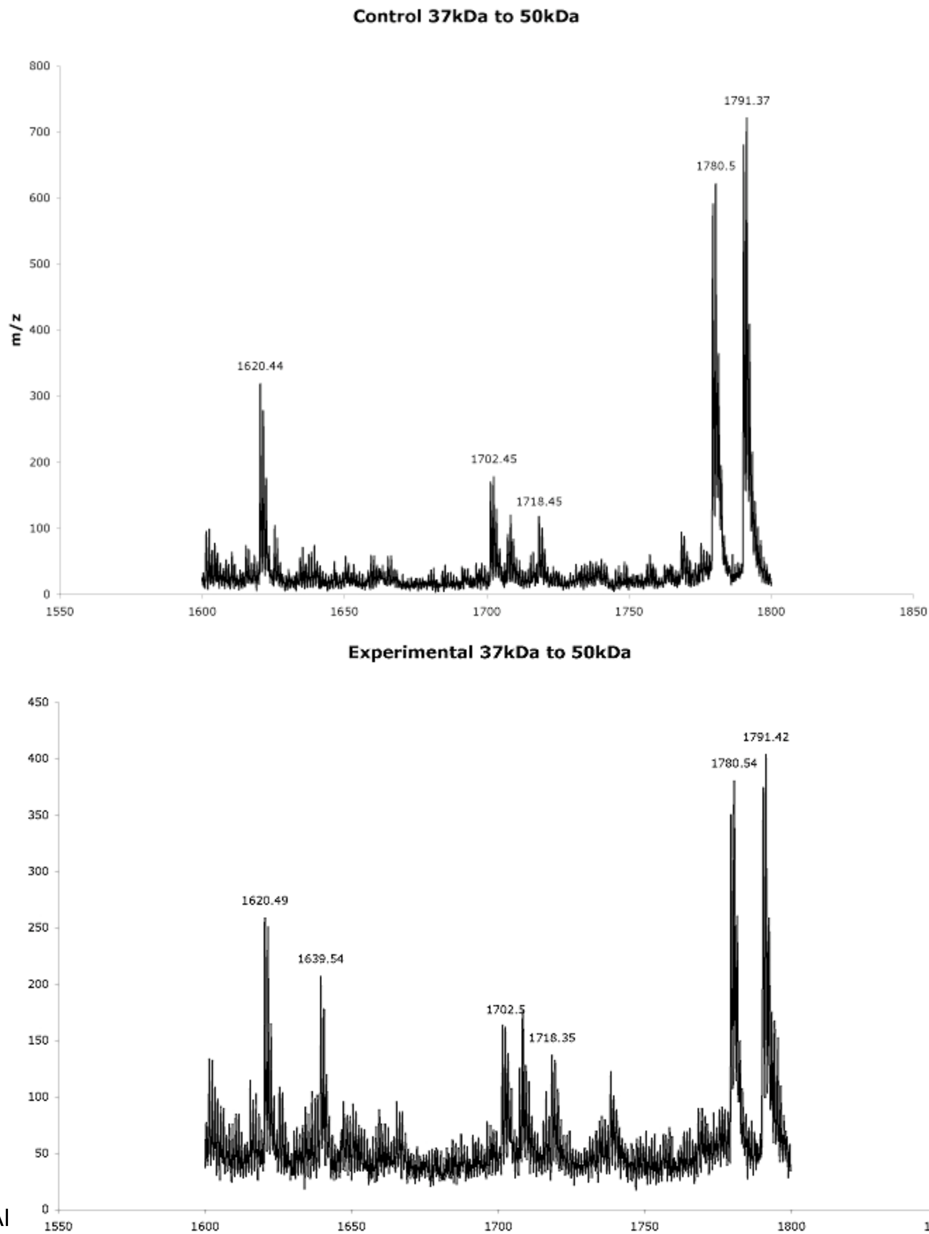

Fig. 1. MAI [illegible] gel. The sham knocl [illegible] des at ~1702.45 may be phosphopeptides, matched by a peptide at m/z ~1620.44. The ratio between the two (0.5 in the control and 0.7 in the experimental) is slightly different here but was also found in replicates of the experiment. In another area of the spectrum (not shown), similar results were found in comparing ~1567.43 and ~1487.54.

We sent these samples on dry ice in 1:1 50% acetonitrile: 50% 0.1% trifluoroacetic acid to the Keck Center. One peptide at ~1701.43 was presumed to be a phosphopeptide, and it was matched by a peptide at m/z ~1620.84. On the MS/MS (tandem mass spectrometry) spectrum we received from the Keck Center, the phosphopeptide was not seen. (Ordinarily, the phosphopeptides are difficult to sequence and so they are only seen if modified techniques are used.) The peptide was isoform 1 of tubulin beta 5 chain. In this region of the MS/MS spectrum, we found 18 peptides from tubulin in the knockdown sample and only 7 in the control and so it is possible that the tubulin is more stable to extraction in the experimental sample.

Another presumed phosphopeptide was at ~1567.43, matching a peptide at ~1487.54. Here, again

MS/MS did not reveal any sequence data in the range of exact mass around the larger peptide, but the peptide itself was a tryptic fragment from isoform 1 of tubulin alpha (1B chain). Therefore, increased stability of tubulin on account of the PKC-epsilon knockdown was a reasonable interpretation of the results. Analysis of the data on tubulin reveals that there is a threonine residue at position 274 of beta5, which can be phosphorylated (NetPhos 2.0 server, Technical University of Denmark), and a serine residue at position 232 of alpha-1B, which can be phosphorylated. Experimental data on rodent cells shows that both are phosphorylated in situ.

We will next introduce phosphopeptides of the same sequence as the two regions represented by the tubulin isoform sequences. This will interfere with the ability of the phosphatase to dephosphorylated the postulated tubulin isoforms and cancel out the effect of PKC-epsilon activaton. If the results of activation rely upon changes in these isoforms, the phenotypic changes typical of cancer cells will be blocked despite the PKC-epsilon being activated with tumor promoter. This will identify a downstream result of PKC-epsilon activation that is clearly relevant to cancer.