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SPOTLIGHT

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Targeting Colon Cancer

Wnt genes encode a family of conserved signaling molecules that regulate cell-to-cell communication during many developmentally critical processes, including embryogenesis, tissue organogenesis, and adult stem cell homeostasis. Abnormal regulation of the Wnt pathway has been associated with cancers in the colon, liver, skin, and breast. Aberrant regulation of β-catenin responsive transcription (CRT), a key transcription activator which itself regulates the Wnt signaling pathway, is a well-documented cause of colorectal cancer. Thus, the identification of compounds that specifically modulate CRT is of great clinical significance. Gonsalves *et al.* (*Proc. Natl. Acad. Sci. U.S.A.* 2011, *108*, 5954−5963) identify three new small molecules that block cell proliferation in cancerous human tumor cells *via* the inhibition of the Wnt signaling pathway.

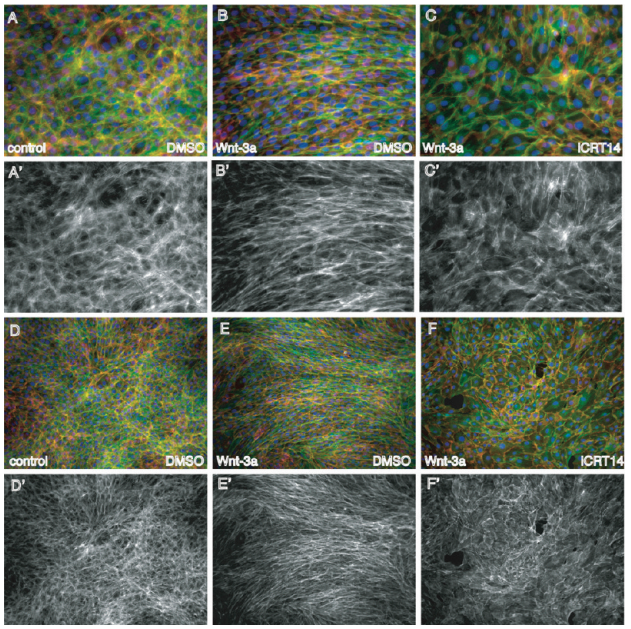
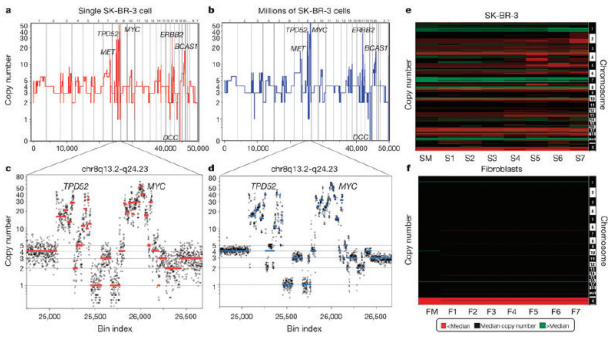


Image credit: Foster Gonsalves.

In this study, the authors used a powerful combination of RNA interference (RNAi)-based screening with a high-throughput chemical genetic screen to study the effects of ~15,000 compounds on the Wnt signaling pathway. Thirty-four compounds were shown to inhibit CRT. Three of these compounds were deemed sufficiently specific to inhibit CRT with minimal to no effect on the noncanonical Wnt pathway or other important growth regulatory pathways, such as JAK/STAT, SHH, and Notch. Biochemical characterization showed that these three inhibitors functioned by inhibiting complex formation of nuclear β-catenin with TCF4 and blocked Wnt/β-catenin-induced target genes and morphological transformations in various mammalian and cancer cell lines. Most remarkably, these small molecule inhibitors were cytotoxic to colon cancer cell lines without affecting the growth and proliferation of healthy cells. The identification of specific inhibitors to the Wnt/β-catenin signaling pathway is an enormous step in the direction of developing better anticancer therapeutics. **Jitesh A. Soares, Ph.D.**

Tumor Evolution, One Cell at a Time

By the year 2020, it is estimated that there will be 16 million new cancer cases and 10 million deaths from cancer worldwide. The prevalence of this devastating group of diseases has prompted much research toward understanding how tumors develop, with most theories revolving around the idea of a gradual progression of cells through various mutated states. Using a technique called single-nucleus sequencing (SNS), Navin *et al.* (*Nature* 2011, *472*, 90−94) examine tumor population structure and evolution in two human breast cancers and present compelling evidence for a distinct tumor progression model referred to as punctuated clonal evolution.



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In SNS, the genomes of individual cells are examined by isolating cell nuclei using flow cytometry methods, amplifying the DNA by whole genome amplification and analyzing the sequence using next-generation sequencing. The authors selected two highly aggressive types of breast tumors for evaluation by SNS. One of the tumors, T10, has been characterized as genetically heterogeneous, or polygenomic, and the other, T16P, as genetically homogeneous, or monogenomic. SNS analysis of T10 indeed revealed the presence of four major distributions of ploidy, or the number of sets of chromosomes, that were anatomically distributed through the tumor. In addition, sequence analysis of 100 cells from T10 revealed four subpopulations, three of which were highly clonal and likely represent three sequential clonal expansions of cancer cells. In contrast, SNS analysis of T16P and a sample from its associated liver metastasis revealed the presence of just two main distributions of ploidy, and analysis of the subpopulations suggested that the primary tumor formed by a single clonal expansion of an aneuploid cell. Notably, analysis of cells from the metastatic tumor suggested that one of the cells from the original expansion seeded the metastasis. On the basis of these results, the investigators propose a model called punctuated clonal evolution, in which tumor subpopulations emerge suddenly and at a rate such that the tumor cell population growth far exceeds the rate of genomic evolution. **Eva J. Gordon, Ph.D.**

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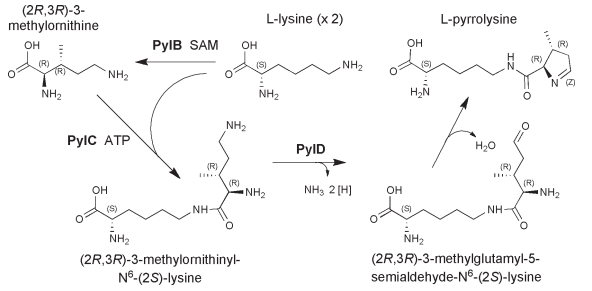
Pyrrolysine Made from Two Lysine Molecules

The 22nd naturally encoded amino acid, pyrrolysine, was discovered nearly a decade ago in methane-producing archaea, as a critical residue in the active sites of methyl transferase enzymes. The organisms incorporate this amino acid using tRNAs that recognize a UAG sequence, normally a stop codon. Previous proposed pathways for pyrrolysine biosynthesis had suggested that other molecules such as ornithine might serve as precursors for the methylated pyrroline carboxylate appended to the lysine side chain. Now Gaston *et al.* (*Nature* 2011, *471*, 647−650) have described how this amino acid is synthesized in the cytoplasm from two molecules of lysine.



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The researchers studied the biosynthesis of pyrrolysine using *E. coli* with genes transformed from two different microbes, *Methanosarcina acetivorans* and *Methanosarcina barkeri*. When they fed the *E. coli* isotopically labeled lysine molecules, mass spectrometry of the digested peptides indicated that the pyrrolysine residues were constructed from two molecules of lysine.

In an earlier study, the addition of D-ornithine to *E. coli* cells that included the genes that facilitate pyrrolysine incorporation stimulated those cells to read through the UAG stop codon. Such cells require all of the three biosynthetic enzymes PylB, PylC, and PylD to incorporate pyrrolysine, but those without PylB are able to incorporate D-ornithine. Gaston *et al.* show that this pathway produces desmethylpyrrolysine, which can be charged onto the tRNA for pyrrolysine and incorporated in proteins.

In addition to explaining pyrrolysine biosynthesis, these results provide a better understanding of the molecular evolution of this amino acid and its position within the aspartic acid-derived family of amino acids. With the exploration of the pyrrolysine and desmethylpyrrolysine biosynthetic pathways, researchers also have valuable clues that would allow them to incorporate synthetic analogues of their biosynthetic precursors into designed recombinant proteins. **Sarah A. Webb, Ph.D.**

RNAi Slips into the Brain

Many laboratories and companies are betting on RNA interference methodologies to transfer into the clinic where mutant mRNA or a virus could be therapeutically marked for destruction. Using systemic siRNAs as a therapy presents a number of difficult hurdles including how to steer these molecules to target only particular cell types. Using RNAi in the brain literally adds another layer of difficulty in the form of the blood-brain barrier, which acts to insulate the brain from many bloodborne molecules. Now, a new method by Alvarez-Erviti *et al.* (*Nat. Biotechnol.* 2011, *29*, 341−345) takes on the mouse brain as an siRNA target with a clever strategy.

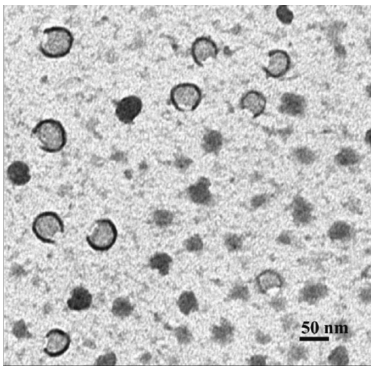
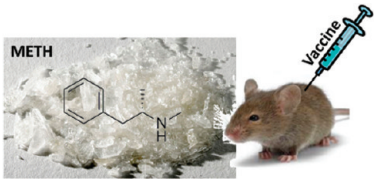


Image Credit: Matthew Wood.

Exosomes, tiny vesicles of 40−100 nm in diameter, are secreted by many cell types and often carry some type of molecular cargo. By isolating and carefully stimulating immature dendritic cells that usually make exosomes, the researchers could produce micrograms of exosomes lacking cargo and subsequently load them with siRNAs instead. Culturing the dendritic cells also allowed for engineered expression of special peptide-tagged versions of the exosome protein, Lamp2b, to effectively target the vesicles to bind either muscle or brain cells. After optimizing the loading of siRNA cargo, the housekeeping gene GAPDH was targeted in cell culture for proof of principle. Exosomes tagged with the muscle peptide effectively knocked down GAPDH in mouse C2C12 myoblasts, while those tagged with a viral peptide that binds the acetylcholine receptor potentiated knockdown in mouse Neuro-2a cells. Then, the siRNA-loaded exosomes were tested *in vivo*, by systemically administering them into a mouse. In this case, a gene relevant to Alzheimer's disease pathology, BACE1, was chosen as the siRNA target. The viral peptide-tagged exosomes effectively reduced the BACE1 mRNA by at least half in the striatum, midbrain, and cortex, three areas rich in acetylcholine receptors. This study indicates an innovative direction for possible siRNA therapy in humans and how exosomes may act as a Trojan horse for getting inside the brain. **Jason G. Underwood, Ph.D.**

A Method Against METH

Methamphetamine addiction is a very serious problem in the United States. In 2009, it was found that over 1 million Americans aged 12 and older had abused the drug at least once during the previous year. Current treatments rely on behavioral therapies, but the high rate of relapse stresses the need for alternate therapeutic strategies. Vaccination, which would initiate the generation of anti-methamphetamine antibodies that would prevent transport of the drug to the brain, is an intriguing approach but has not been met with much success thus far. Moreno *et al.* (*J. Am. Chem. Soc.*, published ASAP ahead of print, DOI: 10.1021/ja108807j) now report their efforts toward the design of an effective new vaccine for methamphetamine addiction.



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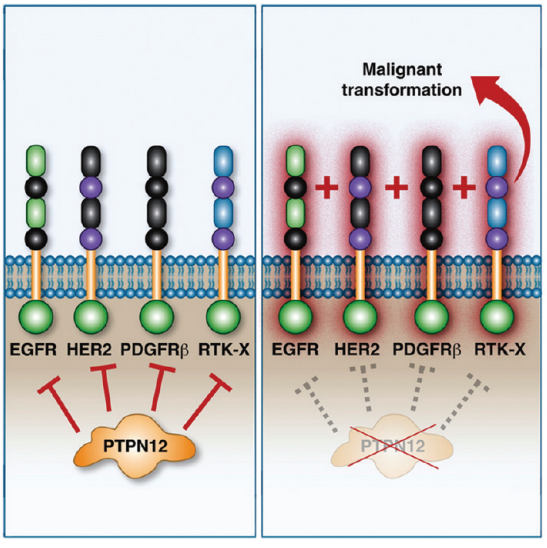
The authors focus on enhancing the three critical factors that contribute to the success of a small molecule vaccine: antibody specificity, affinity, and concentration. First, they use molecular modeling to facilitate the design of a series of six methamphetamine analogues that represent the most stable conformations of the drug and that mimic its most active enantiomer. Key components of their design were the incorporation of molecular constraints, careful attention to stereochemical requirements, and an orthogonal conjugation strategy to maximize the coupling efficiency of the methamphetamine analogue, or hapten, to its protein carrier. The analogues were synthesized and conjugated to protein carriers through a thiol functionality. Mice were vaccinated with the immunoconjugates, and the affinity, specificity, and concentration of the antibodies generated in response to the vaccinations were assessed using a solution-based radioimmunoassay. Of the six compounds, three were particularly promising in that they elicited generation of high concentrations of antibodies that bound (+)-methamphetamine with nanomolar affinity. These results present an exciting new approach for the design of vaccines against addictive drugs that, with further refinement, has tremendous potential for transforming treatment strategies for methamphetamine and other drug addictions. **Eva J. Gordon, Ph.D.**

A Positive Step for Triple Negative Breast Cancer

Although some types of breast cancer respond well to targeted therapies, the subtype referred to as triple negative breast cancer (TNBC) is notorious for the lack of expression of known druggable targets. As TNBC represents approximately one-fifth of breast cancers, there is a great need for characterizing the pathways that drive it. Sun *et al.* (*Cell* 2011, *144*, 703−718) now report the identification of a tumor suppressor network that is compromised in TNBCs, revealing a much needed and perhaps unexpected therapeutic strategy for this elusive type of breast cancer.



Reprinted from *Cell*, *144*, Sun, T., *et al.*, Activation of Multiple Proto-oncogenic Tyrosine Kinases in Breast Cancer via Loss of the PTPN12 Phosphatase, 703-718, Copyright 2011, with permission from Elsevier.

Upon conducting a genetic screen for kinases and phosphatases that suppress transformation of benign human mammary epithelial cells (HMECs) to their malignant counterparts, the protein tyrosine phosphatase PTPN12 emerged as the top candidate. Use of an impressive collection of technologies including protein mutagenesis experiments, quantitative proteomics, and bimolecular fluorescence complementation demonstrated that PTPN12 activity was required for suppression; that PTPN12 suppresses transformation through regulation of the epidermal growth factor receptor signaling network; and that loss of PTPN12 function is correlated with TNBC. In addition, the tumor suppressor REST and the microRNA miR-124 were found to be involved in the posttranscriptional regulation of PTPN12, offering additional clues into the networks that drive TBNC. Finally, it was shown that PTPN12 suppresses tumorigenesis by preventing the activation of several protein tyrosine kinases. Indeed, in a mouse model of TBNC, treatment with a combination of tyrosine kinase inhibitors that target kinases known to be regulated by PTPN12 prevented tumor growth and prolonged survival. Intriguingly, these kinases include the protein tyrosine kinase Her2, whose overexpression in another breast cancer subtype marks those cancer cells for destruction by the successful cancer drug Herceptin. Thus, in TBNC, aberrant regulation of multiple kinases, rather than overexpression of just one, appears to drive tumorigenesis. These findings point to the targeting of multiple kinases under the regulation of PTPN12 as a promising strategy for treatment of TNBC. **Eva J. Gordon, Ph.D.**

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