FIND THE CAUSE
Breast Cancer Foundation

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# Scientific Progress Report

## March 2021

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**Environmental chemicals, the immune system, and predictors of cancer**. While the world thinks about how the immune system can be exploited to beat the SARS-CoV-2 (COVID-19) pandemic, the Find The Cause Consortium has been thinking about how the immune system can be harnessed to prevent or intercept environmental chemical-induced breast cancer before it starts (**Figure 1**). From the total laboratory shutdown last spring, the labs are now up to about 80% capacity as lab personnel become vaccinated. With the establishment of a new normal in sight, the labs have returned to long-planned and long-term studies using multiple models of breast and other cancers and computational methods to determine the plausibility of: 1) detailing how environmental chemicals contribute to cancer, 2) identifying cells destined to become cancerous after environmental carcinogen exposure, and 3) determining practical methods for enhancing the immune responses with the goal of intercepting cancer before it happens, i.e., secondary prevention. This work has only gained in immediacy as the number of chemicals registered by the EPA has risen to 86,557 as of March, 2021.

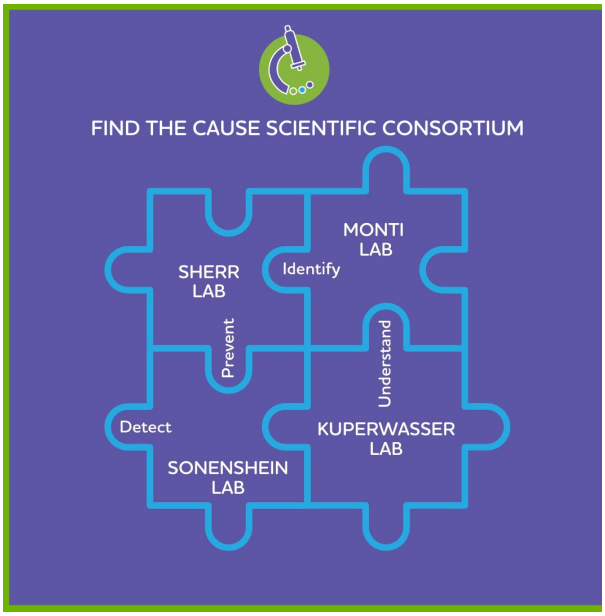


**Figure 1. Prevent (Primary prevention):** Identify what environmental chemicals are carcinogens and eliminate them**; (Secondary prevention):** Detect who has been exposed, predict impending cancers, intercept at least by enhancing cancer immunity; **Understand:** Learn how cancers happen, identify their vulnerabilities; extrapolate to how environmental chemicals contribute to breast cancer.

**Monti lab**. Work in the Monti lab over the past six months has encompassed computational tool development and application of those tools to the study of breast cancer's mechanisms of action. A deeper understanding of the cross-talk between fully malignant cells and the microenvironment in which they exist in breast tissue is essential for elucidating mechanisms of cancer initiation, progression and sensitivity to intervention approaches. A large component of that tumor microenvironment is composed of immune cells that have migrated to the tumor site in the immune system's attempt to kill the tumor. The level of immune cell infiltration and the makeup of the types of immune cells in the tumor, to a very large extent, determines

whether the tumor grows, dies or is maintained at an acceptable level. In a recent study currently under review at the *Nucleic Acids Research* journal, the Monti lab applied *K2Taxonomer*, a computational tool that it recently developed with Find The Cause funding, to the analysis of the genomes of immune cells infiltrating human breast cancers on a single-cell basis. This type of analysis of individual cells represents a cutting edge technology in cancer biology and has the potential to reveal how each immune cell behaves as it respond to the cancer. The Monti lab analysis defined a dominant set of genes activated by immune cells (T cells) that is common to multiple immune T cell subsets within the cancer. Importantly, the expression of this gene set defines a subgroup of tumor-infiltrating lymphocytes (white blood cells in the immune system, aka TILs) and is associated with better survival. In essence, these studies point to what the immune system has to do in order to successfully kill the cancer. Not only does this allow us to better predict how breast cancer patients will fare after diagnosis, but also reveals what specific immune programs need to be enhanced to generate a (literally) killer immune response.

In another study, the Monti lab applied another novel computational tool to rapidly analyze commonalities and differences between breast cancers from hundreds of patients in a publically available database located at the National Cancer Institute and known as The Cancer Genome Atlas (TCGA). The ongoing analysis is identifying important networks of highly connected genes in different breast cancer subtypes (e.g., Luminal A *vs*. Basal cell cancers). These studies are beginning to reveal which genes are driving the different breast cancer types. A representation of cancer genes and their interactions with one another is provided in **Figure 2**.

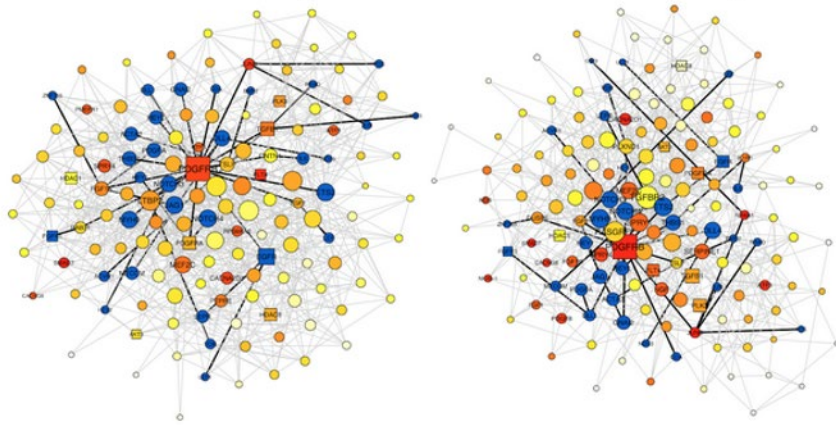


**Figure 2.** Schematic showing the interactions of multiple genes in two different types of breast cancer, Luminal A and the somewhat more aggressive Basal-like breast cancers. A common gene likely to be important in driving both types of breast cancers codes for a cell growth factor (PDGFB). Note the PDGFB gene in the red rectangles at the center of both of these webs of interacting cancer genes.

**Sherr lab**. In collaboration with Drs. Meriem Koual and Xavier Coumoul at the University of Paris and the French National Institute of Health (INSERM) respectively, the Sherr lab published a manuscript last month demonstrating that even malignant breast cancer cells need help growing and surviving in what otherwise would be considered a hostile (immune) environment. The studies show that breast cancer cells, exposed to common environmental chemicals that activate the aryl hydrocarbon receptor (AhR), get a boost from surrounding cells of the fat cell lineage. Indeed, the data suggest that the ability of fat cells to store high levels of environmental chemicals contributes to the adverse interactions between fat cells and those cells destined to become malignant cancerous cells. These studies are related to those of Dr. Monti (above) in

that they illustrate how the local cancer microenvironment contributes to malignant cell growth. The results also build on previous studies from the Sherr lab that showed, for the first time, that environmental chemicals can drive breast cells to assume characteristics of breast cancer stem cells, a cancer cell subtype that is thought to be responsible for cancer metastasis and re-emergence after remission. One implication of these studies is that AhR inhibitors, such as those developed by the Sherr lab, could one day be used to shut down the conversation between fat lineage cells and malignant cells. The work was published in the leading journal of the National Institute of Environmental Health Sciences (*Environmental Health Perspectives*).

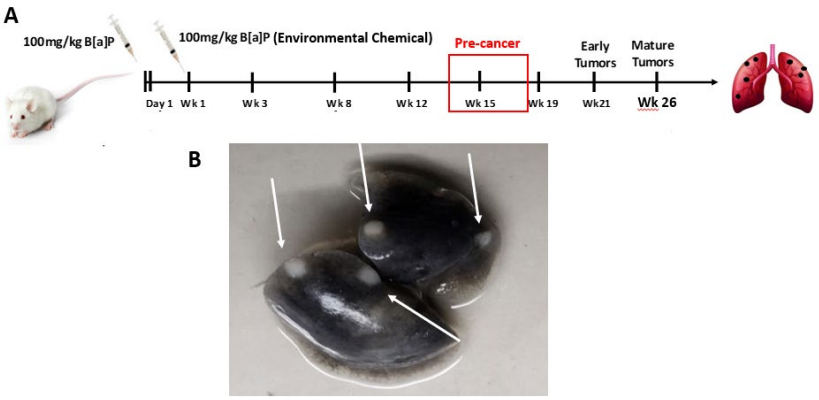


**Figure 3**. **A)** Protocol for environmental chemical-induced lung cancer. Carcinogen (B[a]P) was injected on days 0 and 7. At 14 weeks, when molecular changes presaging full-blown lung cancers were seen, mice were treated with an AhR inhibitor given daily by mouth. Lungs were collected at week 26 and lung tumors quantified. **B)** White tumors stand out in lungs stained with a black dye (white arrows).

The Sherr lab also as completed the harvesting of tissue from mice exposed to a common environmental chemical found in cigarette smoke and ambient air pollution (B[a]P). This chemical is similar to that which routinely induces breast cancers in rats and mice (another polycyclic aromatic hydrocarbon called DMBA) and studied previously in the Sherr lab. The lab determined that mice treated with B[a]P develop multiple lung cancers 26 weeks later (**Figure 3**). In preliminary studies, the lab generated data suggesting that carcinogen-treated mice in which prophylactic treatment with an AhR inhibitor was begun before overt tumor formation (at 14 weeks) generated less than half as many tumors as mice not treated with AhR inhibitor. These results suggest that cancer can be intercepted by blocking the AhR. The lab's initial analysis of immune cells that have infiltrated the lungs of mice treated with carcinogen strongly suggests that preventative treatment with AhR inhibitor significantly increases the number of cancer-killing immune cells (killer T cells) by greater than 20 fold. While very preliminary, these studies are encouraging and motivate molecular analysis of markers of cancer prevention (see below) and further studies in this model of primary, environmental chemical-induced cancer.

Finally, in a study published in the high-ranking journal, *Nature Neuroscience*, Dr. Sherr's collaborators demonstrated that the AhR plays a key role in the early critical immune responses to viruses like Zika and Dengue virus. Indeed, the AhR inhibitors developed in the Sherr lab significantly reduced Zika virus infection in cells in culture and, when given to pregnant Zika virus-infected mice, blocked Zika-mediated developmental defects seen in pups after birth.

These results open an entirely new window into how this environmental chemical receptor contributes to immunity in multiple contexts.

**Sonenshein Lab**. Dr. Sonenshein's laboratory continues to work on the development of monoclonal antibodies capable of binding a surface protein on breast cancer cells called "Adam-8". In studies over the last five years the lab has shown that these antibodies are effective at reducing cancers in mice. In theory, they also could be used to block development of new (secondary) cancers either after initial cancer treatment or prior to formation of primary tumors.

The lab also has been working with Dr. Monti to determine the feasibility of screening blood for markers of pre-cancers. They have settled on a type of nucleic acid related to DNA that may persist in the blood revealing when molecular changes consistent with pre-cancer have happened. These "microRNAs" are generally associated with gene suppression and may inhibit expression of genes that control the immune system or that block growth inhibition signals within malignant cells. The Sonenshein lab is now attempting to purify these microRNAs from tumor-bearing lung tissue and from the blood of mice treated with environmental carcinogen (B[a]P) with and without AhR inhibitor in the Sherr lab experiment described above. The goal is to determine if a profile of microRNA expression in the lung and in blood can be used to mark an early pre-cancer period. This test could be used to determine when interception, for example with cancer preventatives like AhR inhibitors or Adam-8-specific antibodies, should begin.

**Kuperwasser Lab**. In experiments that parallel those described from Dr. Sonenshein's laboratory, Dr. Kuperwasser's lab is purifying DNA from lung and blood samples from the carcinogen ± AhR inhibitor-treated mice described above to determine if DNA from cancer, pre-cancer, or simply environmental chemical-exposed normal tissue bears tell-tale "epigenetic" marks on DNA that can be used to determine if an individual has been exposed to environmental chemicals or if changes consistent with pre-cancer have happened. These studies are based on the emerging field of epigenetics in which environment-induced changes in DNA structure are shown to be fixed and can be tracked for long periods of time. A long-term goal of these studies is to determine if epigenetic changes can be used to determine chemical exposures that may have happened years earlier.

In a second set of experiments related to the immunological work in Dr. Sherr's lab, Dr. Kuperwasser's lab is staining thin sections of environmental chemical-induce cancers to determine what kinds of immune cells have infiltrated the tumor and where they are located relative to one another. This work dove-tails with the Sherr lab quantifying immune cells in the tumor microenvironment. In the Sherr lab work, the numbers of cell types, but not their geographical location within the tumor, can be determined. The Kuperwasser lab studies are expected to plug that gap in our knowledge.

**Presentations**

**Sherr DH**. *The Role of the Aryl Hydrocarbon Receptor and the Kynurenine Pathway in Cancer Immuno-Metabolism*. Discovery on Target Conference. September 16-18, 2020. Boston, MA. (Virtual).

**Sherr DH**. *The Aryl Hydrocarbon Receptor: A Master Immune Checkpoint Regulator?.* 2020. Dana Farber-Boston University Head and Neck Cancer Symposium Boston, MA. (Virtual)

**Sherr, DH**. *Intercepting the AhR to Enhance Tumor Immunity and Prevent Lung Cancer*. Johnson & Johnson Lung Cancer Initiative, March 2021.

**Monti S**. *The Xposome – Integration and Sharing of Xenobiotics-Associated Assays across Species, Phenotypes, and Sites*. Superfund Research Program External Use Cases Showcase. National Institute of Environmental Health. February 2021.

**Monti S**. *Modeling Chemicals' Adverse Effects by high-throughput transcriptomics*. Exposure and Latent Disease Risk eLearning Web Seminars: Session IV - Moving Forward. National Institute of Environmental Health. June 2021

**Full-length Manuscripts and Publications, July, 2020-March, 2021**

Kenison, JE, Jhaveri A, Li Z, Khadse N, Tjon E, Tezza S, Nowakowska D, Plasencia A, Stanton VP, **Sherr DH**, and Quintana FJ. 2020. *Tolerogenic nanoparticles suppress central nervous system inflammation. Proc Natl Acad Sci U S A*.

Wang, Z, Snyder M, Kenison JE, Yang K, Lara B, Lydell E, Bennani K, Novikov O, Federico A, **Monti S**, and **Sherr DH**. 2020. *How the AhR Became Important in Cancer: The Role of Chronically Active AHR in Cancer Aggression*. *Int J Mol Sci* 22.

Giovannoni F, Bosch I, Polonio CM, Torti MF, Wheeler MA, Li Z, Romorini L, Rodriguez MS, Rothhammer V, Barroso A, Tjon EC, Sanmarco LM, Takenaka MC, Modaresi SMS, Gutierrez-Vazquez C, Zanluqui NG, Dos Santos NB, Munhoz CD, Wang Z, Damonte EG, **Sherr DH**, Gehrke L, Peron JPS, Garcia CC, and Quintana FJ. 2020. *AhR is a Zika virus host factor and a candidate target for antiviral therapy. Nat Neurosci* 23: 939-951.

Koual M, Tomkiewicz C, Guerrera IC, **Sherr DH**, Barouki R, and Coumoul X. 2021. *Aggressiveness and Metastatic Potential of Breast Cancer Cells Co-Cultured with Preadipocytes and Exposed to an Environmental Pollutant Dioxin: An in Vitro and in Vivo Zebrafish Study*. *Environ Health Perspect* 129: 37002.

Kenison-White J, Wang Z, Yang K, Snyder M, Quintana FJ, and **Sherr DH**. 2021. *The aryl hydrocarbon receptor suppresses immunity to oral squamous cell carcinoma through immune checkpoint regulation*. Under review.

Reed ER & **Monti S**. (2020). *Multi-resolution characterization of molecular taxonomies in bulk and single-cell transcriptomics data*. BioRxiv, 2020.11.05.370197. (Under 2nd review at Nucleic Acid Research) https://doi.org/10.1101/2020.11.05.370197

Federico, A., Klein, J., Sebastiani, P., Varelas, X., & **Monti, S**. (2021). *Hierarchical Reconstruction of Regulatory Networks for the Identification of Candidate Cancer Drivers*. Manuscript in Preparation.