Liron Bar-Peled, PhD

Bar-Peled Laboratory

Yousuf Ali, PhD
Liron Bar-Peled, PhD
Nicholas Chen*
Harrison B. Chong
Zhuanglin Dai*
Lina Fellah*
Jay-Miguel Fonticella*
Maolin Ge, PhD
Magdy Gohar
Mariko Hara, PhD
Stefan Harry, PhD
Yingfei Huang
Neha Khandelwal, PhD
Matthew Lazarov
Chau Le
Christine Lee
Seungjun Lee
Farah Makram
George Popoola
Patrick Tein, PhD
Samay Trivedi
Janice Wong
James Woods
Junbing Zhang, PhD
Siwen Zhang, PhD

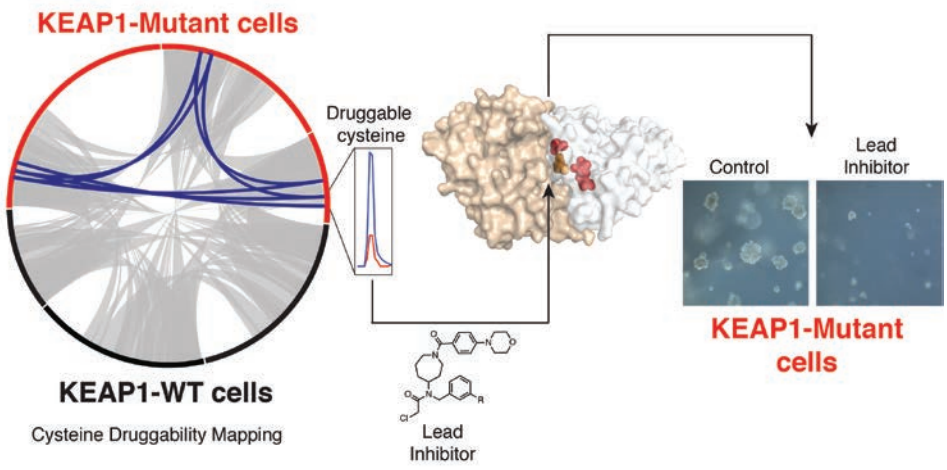
*Graduate student

Research in **the Bar-Peled laboratory** sits at the interface of cellular metabolism and signal transduction and focuses on understanding how cancer cells respond to altered metabolic states. Rapidly proliferating cancer cells are characterized by increased production of toxic metabolic byproducts known as reactive oxygen species (ROS) that at high levels potently block cancer cell growth. To neutralize high ROS levels, cancer cells activate the NRF2 pathway, which governs the cellular antioxidant response. While the NRF2 pathway is critical for cancer growth, the molecular mechanisms by which this pathway functions and provides cancer cells with a proliferative advantage remain poorly understood. By combining frontier molecular, chemical and proteomic approaches, research in our lab has revealed that NRF2 establishes a unique cellular environment that protects critical proteins required for cancer cell growth from inactivation by ROS. Our studies indicate that these ROS-regulated proteins are highly targetable by small molecule inhibitors and may be exploited to develop chemical tools to inactivate these dependencies in cancers.

Cancer cells display remarkable plasticity allowing them to adapt to ever changing environments. A key feature of this plasticity is their ability to rewire core metabolic networks to provide a steady source of energy and building blocks needed for rapid growth. This demand for energy produces byproducts, including ROS that alters the function of proteins, DNA and lipids, and if left unchecked, results in oxidative stress and impairs cancer cell viability. To counter a rise in oxidative stress, cells activate the NRF2 transcription factor leading to the expression of a vast network of antioxidant and detoxification genes that restore redox homeostasis. Multiple cancer cells, including ~30% of non-small cell lung cancers (NSCLCs) activate NRF2 through the genetic disruption of its negative regulator KEAP1. Despite its clear importance in cancer cell proliferation, we know remarkably little about how the NRF2/KEAP1 pathway functions within cancer cells or how ROS modification of proteins alters their function. Our long-term goal is to understand how cancer cells sense and respond to ROS and to pharmacologically modulate these pathways in cancers where they are deregulated.

Redox control pathways in lung cancer

Our recent studies focus on how the intracellular environment generated by NRF2 in NSCLCs is required for cancer cell proliferation. By employing a chemical proteomics platform (isoTOP-ABPP) that identifies changes in cysteine reactivity mediated by ROS, we demonstrated that NRF2 is required for the protection of dozens of proteins from ROS modification. We found that silencing NRF2 in NSCLCs reduced the reactivity of the catalytic cysteine of the glycolytic enzyme GAPDH without changing GAPDH protein abundance. Concomitant knockdown of NRF2 significantly reduced GAPDH enzyme activity and glycolytic flux, a metabolic pathway required to fuel cancer cell proliferation. These results illustrate how NRF2 can regulate enzyme and pathway activity, not through direct transcriptional control, but rather by fostering a favorable redox environment required for proper



(Left) A cysteine druggability map identifies proteins exclusively druggable in KEAP1-mutant NSCLC cells enabling the development of small molecule inhibitors that disrupt NR0B1 protein interactions (middle) and block KEAP1-mutant cell growth (right).

Images from Bar-Peled et al., 2017.

enzyme function. Current studies in our lab seek to elucidate how other proteins are post-translationally regulated by NRF2 and feedback into this pathway. To address these questions, we are studying the function of ROS-regulated sites on proteins as well as the identifying reactive metabolites that modify them.

Druggable co-dependencies

Our investigations suggest that the cellular state created by NRF2 may be exploited to develop inhibitors targeting proteins whose expression and function are stimulated by this environment. Because of their importance to protein function, cysteines are targeted by multiple clinically approved inhibitors. To identify pharmacological targets of the NRF2 pathway, we use powerful chemical proteomic platforms (cysteine druggability mapping) to identify the landscape of protein druggability (e.g. ligand-protein interactions) in genetically defined lung cancers. Our studies reveal that multiple proteins, including the orphan nuclear receptor NR0B1, are exclusively druggable in KEAP1-mutant, NRF2-activated cells. By developing a small molecule inhibitor that disrupts NR0B1 protein interactions we show that NR0B1 functions as a critical signaling node within the NRF2 pathway to support its pro-proliferative transcriptional output required for anchorage-independent growth. Recently we uncovered that cysteine residues that are sensitive to ROS modification are highly targetable by covalent inhibitors. Our current studies suggest that these sites may be exploited to develop inhibitors that target proteins required for the proliferation of NRF2- activated cancers.

Ongoing projects:

1. Determine how cancer proteomes respond to changes in the intracellular redox environment
2. Elucidate the role of NRF2-regulated reactive metabolites on protein function
3. Decipher how cells adapt to anchorage-independent growth
4. Identify druggable transcriptional dependencies in genetically-defined cancers

Selected Publications:

Weiss-Sadan T, Ge M†, Hayashi M, Gohar M, Yao CH, de Groot A, Harry S, Carlin A, Fischer H, Shi L, Wei TY, Adelmann CH, Wolf K, Vornbäumen T, Dürr BR, Takahashi M, Richter M, Zhang J, Yang TY, Vijay V, Fisher DE, Hata AN, Haigis MC, Mostoslavsky R, Bardeesy N, Papagiannakopoulos T, **Bar-Peled L†**. NRF2 activation induces NADH-reductive stress, providing a metabolic vulnerability in lung cancer. *Cell Metab*. 2023 Apr 4;35(4):722.

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*These authors contributed equally to this work

†Co-corresponding authors