Unfolding triple-negative breast cancer

By Benjamin Boettner, Associate Editor

A team from **Weill Cornell Medical College** has found a unifying feature of triple-negative breast cancers—overactivation of the transcription factor X-box binding protein 1—that could open the door to new therapies for this notoriously hard-to-treat disease.¹ Although blocking expression of this target, which is involved in the unfolded protein response, decreases tumor formation and relapse in mice, more druggable targets upstream of it might be better suited for further development.

Triple-negative breast cancers lack expression of estrogen receptor, progesterone receptor and HER2 (EGFR2; ErbB2; neu) and thus do not respond to hormonal or HER2-directed therapy.²

Instead, patients receive chemotherapy—which typically produces a good initial response. However, a significant proportion of patients relapse, and the cancer often metastasizes.

Triple-negative cancers represent about 10%–20% of invasive breast cancers and are among the most aggressive types of breast cancer.

In 1999 Laurie Glimcher—who was then at the **Harvard School of Public Health**—coauthored a transcriptional study on multiple myeloma (MM) that showed upregulation of X-box binding protein 1 (XBP1), a protein associated with a cellular stress response to improper protein folding, in this cancer.³ Results from other labs corroborated the findings in MM and further showed involvement of the pathway in various other tumors.⁴

Now, Glimcher and colleagues have tied the unfolded protein response to triple-negative breast cancer.

Glimcher is dean and a professor of medicine at Weill Cornell.

The team also included researchers from the **University of California, Los Angeles, The University of North Carolina at Chapel Hill, Tongji University, Sichuan Agricultural University, Brigham and Women's Hospital, Boston Children's Hospital** and the **Dana-Farber Cancer Institute**.

X-box on

The unfolded protein response is part of the cellular adaptive response to stress. In normal cells the response is activated when improperly

folded proteins accumulate in the endoplasmic reticulum following exposure to stimuli such as hypoxia.

A key player in the pathway is endoplasmic reticulum to nucleus signaling 1 (ERN1; IRE1), a kinase and endoribonuclease in the endoplasmic reticulum membrane that senses misfolded proteins. Activated IRE1 splices a short inhibitory sequence out of the *XBP1* mRNA transcript, which triggers translation of *XBP1* to an active transcription factor that turns on a battery of genes to normalize protein folding.

To see what role XBP1 plays in different types of breast cancer, the researchers scanned a panel of breast cancer cell lines for the activation status of *XBP1*. They discovered that the spliced, active form was on average about twice as prevalent in triple-negative breast cancer cell lines than other breast cancer cell lines.

Next, the team used shRNA to deplete *XBP1* mRNA in a mouse xenograft model of triple-negative breast cancer and found that *XBP1* knockdown decreased tumor growth, angiogenesis and metastasis to the lung compared with no alteration.

shRNA-mediated *XBP1* knockdown also interfered with tumor formation and extended survival in mice transplanted with patient-derived triple-negative primary cells.

Glimcher's group then investigated whether XBP1 is connected to the high relapse rate in triple-negative breast cancer.

By combining the chemotherapeutic doxorubicin with shRNA-mediated inhibition of *XBP1*, the researchers produced greater growth inhibition in the patient-derived cells than that seen using either agent alone and prevented or delayed relapse of the tumors in mice.

In triple-negative breast cancer, relapses can be triggered by tumor-initiating cells that have a characteristic CD44^{high}/CD24^{low} surface marker expression.

Silencing *XBP1* decreased the fraction of CD44^{high}/CD24^{low} in a population of transformed breast cells and interfered with their potential to form mammospheres, a readout for tumor-initiating potential.

Finally, the team searched for genes regulated by *XBP1* activation in triple-negative cells and identified *hypoxia-inducible factor 1α* (*HIF1A*; *HIF1α*) as a pivotal component of

XBP1's tumorigenic pathway.

An extensive chromatin immunoprecipitation analysis coupled with high throughput sequencing (ChIP-seq) revealed XBP1 binding sites at a significant number of loci that coincided with binding sites for HIF1α.

Other studies had previously linked hyperactivated HIF1α with triple-negative breast cancer and had shown it to be required for maintenance of the CD44^{high}/CD24^{low} cell signature.^{5–7}

Here, Glimcher's study connected XBP1, HIF1α and tumor formation by showing that XBP1 reinforced HIF1α activity in mammospheres *in vitro* and in triple-negative breast cancer xenografts *in vivo*.

The team concluded that *XBP1* activation promotes tumor formation in triple-negative breast cancers by acting through HIF1α.

The findings were published in *Nature*.

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—Connie Eaves, BC Cancer Agency

Unfolding targets

Glimcher told *SciBX* that the next step is to extend the studies' validation using multiple preclinical models with patient-derived xenografts representing major triple-negative breast cancer subtypes and with genetically engineered mouse models bearing mutations seen in human triple-negative breast cancers.

Connie Eaves agreed that the interesting findings need additional validation because the data in Glimcher's paper were based on breast cancer cell lines and only one patient's tumor.

"It will be crucial to examine the pathway and the effects of its manipulation in primary tumor cells from multiple patients including a full spectrum of triple-negative breast cancers and non-triple-negative cancers," she said.

"While XBP1 is a transcription factor, and traditionally transcription factors have been difficult to target with small molecules, IRE1 is a viable drug target."

—Laurie Glimcher,
Weill Cornell Medical College

Eaves is a professor of medical genetics at the **BC Cancer Agency's** Terry Fox Laboratory and works on hematopoietic and breast stem cells in cancer.

In addition, Glimcher and Eaves agreed that the next step is to identify the optimal therapeutic target in the XBP1 pathway.

Glimcher told *SciBX*, "While XBP1 is a transcription factor, and traditionally transcription factors have been difficult to target with small molecules, IRE1 is a viable drug target." She added that her team is planning to study IRE1 inhibitors in preclinical and genetic models.

Eric Chevet agreed that IRE1 could be a good target to investigate in triple-negative breast cancer. He added that several IRE1 inhibitors are in development.

Chevet is a researcher at the **Institut National de la Santé et de la Recherche Médicale (INSERM)** at the **University of Bordeaux Segalen**. He is studying the unfolded protein response in normal and pathological processes.

MannKind Corp. has the IRE1 inhibitors MKC204 and MKC-3946 in preclinical testing for MM. **Ruga Corp.** has STF-08310, an IRE1 inhibitor, in preclinical testing for MM and breast cancer.

However, Chevet cautioned that IRE1 has other activities in addition to XBP1 splicing. It helps degrade a pool of mRNAs in a process called regulated IRE1-dependent decay (RIDD) and cleaves premature microRNAs. Both these activities "can have proapoptotic effects that may run opposite to survival-promoting XBP1 splicing," he said.

"Finding agents that uncouple IRE1's XBP1 from its RIDD activity could give the overall best net effects. So far, no small molecules with this potential have been described, but peptide studies have shown that this is possible," he added.

Ruga CEO Raymond Tabibiazar agreed that IRE1's multiple activities make it a challenging pharmacological target.

"It is also known that proteasome inhibitors like Velcade bortezomib increase the unfolded protein response," he said. "Thus, it is tempting to speculate what happens if triple-negative breast cancers are treated with proteasome inhibitors. Would this push them over the edge and kill them?"

That approach, according to Tabibiazar, would be opposite to inhibiting XBP1. Instead, it would amplify XBP1 activation—and theoretically produce the same result.

Chevet also noted, "Another potentially very specific target could be the elusive ligase that completes the IRE1-initiated splicing reaction on XBP1, which so far has not been identified."

Glimcher told *SciBX* that her lab is exploring the upstream signals that activate the IRE1- and XBP1-controlled sensor mechanism in response to hypoxia in triple-negative breast cancer cells, which could reveal additional therapeutic entry points.

Weill Cornell Medical College has filed a patent application on the findings. The licensing status is unavailable.

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