Text by Caitlin Sedwick
csedwick@gmail.com

Glycolytic cancer cells splice their way out of trouble

Cancer cells' glycolytic phenotype drives alternative splicing of the proapoptotic protein Bnip3, producing a splice variant that protects against death.

Focal Point

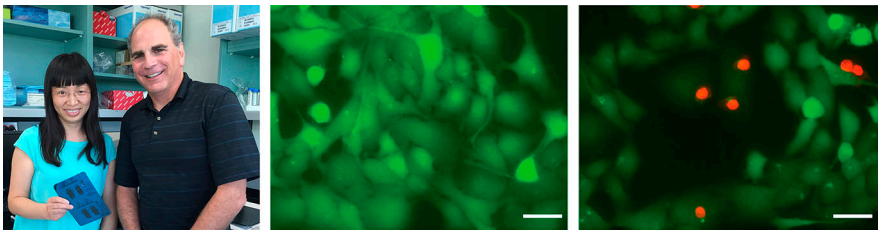

PHOTO COURTESY OF LORRIE KIRSHENBAUM

Hongying Gang (left), Lorrie Kirshenbaum (right), and colleagues found that glycolytic cancer cells express high levels of an alternatively spliced version of Bnip3 that suppresses the proapoptotic functions of the full-length protein. Hypoxic cancer cells lacking the splice form (right) are therefore more susceptible to death (red cells) than controls (left).

Most human cells utilize both glycolysis and oxidative respiration to generate the energy they need. Although oxidative respiration is more efficient, glycolysis does not require oxygen and therefore tends to predominate in times of extreme environmental stress. For example, cardiac myocytes will fall back on glycolysis in the hypoxic environment that arises after infarct. This may not be enough to sustain the tissue; if the damage is too severe, cells are driven to apoptosis. Cancer cells, however, are often resistant to apoptosis even when placed under severe metabolic stress.

A notable feature of many cancers is their reliance on glycolysis rather than oxidative respiration for energy generation. This may originate as a way to cope with the poorly vascularized, hypoxic environment within solid tumors, but cancer cells tend to rely on glycolysis even when oxygen is present. What advantage cancer cells gain from this metabolic switch—named the Warburg effect after Nobel laureate Otto Warburg, who was the first to observe it (1)—remains a mystery. Now, Gang et al.offer new insights into how cancer cells' glycolytic phenotype helps facilitate their remarkable resilience to metabolic stress (2).

> **"So the question was, if we remove the [Bnip3] splice variant, can we sensitize cancer cells to death?"**

Some cancers sport mutations in pro- or antiapoptotic genes, but others don't, yet still survive under conditions that would kill normal cells. An early step in apoptosis is the permeabilization of the mitochondrial outer membrane by proapoptotic Bcl-2 family proteins. For example, Lorrie Kirshenbaum and colleagues at the University of Manitoba in Winnipeg previously demonstrated that the Bcl-2 family protein Bnip3, which is strongly upregulated by hypoxic conditions, drives apoptosis in hypoxic cardiomyocytes (3). This may explain the high risk of heart failure associated with the widely used cancer drug, doxorubicin; the drug stimulates Bnip3 translocation to mitochondria and induces cardiomyocyte necrosis (4). But Kirshenbaum's group also noticed that an alternatively spliced form of Bnip3 is up-regulated in cardiomyocytes exposed to hypoxia (5). This splice variant lacks the gene's third exon and suppresses the full-length protein's proapoptotic activities, thereby preventing excessive cardiomyocyte death.

Upon surveying several cancer cell lines and cancer patient–derived cells, Research Associate Hongying Gang and her colleagues in Kirshenbaum's lab observed that the shorter splice variant was the predominant Bnip3 isoform expressed in cancers. Hypothesizing that Bnip3 splicing may be linked to the cells' metabolic phenotype, the researchers restored oxidative respiration by interfering with the glycolytic enzyme PDK2, which is upregulated in cancer cells, particularly under hypoxic conditions. Inhibition or knockdown of PDK2 blocked Bnip3 alternative splicing, whereas supplementing cells with pyruvate (the metabolic product of glycolysis) enhanced production of the splice form. Therefore, the glycolytic phenotype of cancer cells drives alternative splicing of Bnip3.

"So the question was, if we remove the splice variant, can we sensitize cancer cells to death by hypoxia, to doxorubicin, or to full-length Bnip3 itself? The answer was yes," says Kirshenbaum. When not countered by the shorter splice variant, full-length Bnip3 efficiently killed cancer cells. This contrasts with earlier reports that overexpression of Bnip3 in cancer cells drives autophagy, a process that helps cells survive energetic privation (6). However, Gang et al. observed that, in fact, autophagy only occurs in cancer cells overexpressing full-length Bnip3 when the splice form is also expressed. The splice form, whose existence was then unknown, may have thrown off those earlier studies.

Therefore, this study suggests that cancer cells' glycolytic phenotype drives alternative splicing of Bnip3, which protects them from death caused by full-length Bnip3. Is the Bnip3 splice variant a kind of Achilles' heel for cancer cells? Kirshenbaum's group is currently working to understand all the protein's functions in cells. They also aim to pharmacologically target the splice form in hopes that this may impair cancer growth, possibly in conjunction with doxorubicin treatment.

1. Warburg, O. 1956. *Science*. 123:309–314.
2. Gang, H. et al. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201504047
3. Regula, K.M., et al. 2002. *Circ. Res.* 91:226–231.
4. Dhingra, R., et al. 2014. *Proc. Natl. Acad. Sci. USA*. 111:E5537–E5544.
5. Gang, H., et al. 2011. *Circ. Res.* 108:1084–1092.
6. Mazure, N.M., and J. Pouysségur. 2009. *Autophagy*. 5:868–869.