Glutathione Metabolism: An Achilles' Heel of ARID1A-Deficient Tumors

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In this issue of *Cancer Cell,* Ogiwara et al. describe a novel link between the epigenetic regulator ARID1A and glutathione metabolism in cancer that is mediated by regulation of the cystine/glutamate transporter XCT. This work reveals that synthesis of reduced glutathione is a metabolic dependency of cancers with *ARID1A*-inactivating mutations.

Epigenetic regulators determine gene expression by shaping the structure and accessibility of chromatin. ARID1A/BAF250A is a subunit of the SWI/SNF chromatin remodeling complex, which uses the energy of ATP hydrolysis to remodel chromatin structure (Kadoch and Crabtree, 2015). Investigations of the ARID1A gene mutations and its protein and mRNA levels have shown that ARID1A loss occurs in a large spectrum of cancers at up to 50% frequency (Wu and Roberts, 2013). The mechanism of tumor suppression by ARID1A has been mainly attributed to its role within the SWI/SNF complex, and loss of ARID1A results in changes to cellular proliferation, differentiation, and apoptosis that have tumorigenic consequences.

Ogiwara et al. now report a role for ARID1A in the maintenance and promotion of glutathione metabolism in cancer cells (Ogiwara et al., 2019). The discovery of this link has important therapeutic implications for the treatment of ARID1A-deficient tumors, which are more sensitive to inhibitors of reduced glutathione (GSH) synthesis such as buthionine sulphoximine (BSO), PRIMA-1, and its analog APR-246. To understand the mechanistic basis of this sensitivity, Ogiwara et al. analyzed gene expression profiles in a panel of human cancer cell lines (Ogiwara et al., 2019). For each tumor type, gene expression patterns were compared in two cell lines that differed in the mutational status of *ARID1A*. Among over 300 genes perturbed by ARID1A loss, only *SLC7A11* in the GSH metabolic pathway was consistently affected in all ARID1A-deficient cell lines. The authors then demonstrated that ARID1A occupies the transcription start site (TSS) of *SLC7A11* along with BRG1, the catalytic subunit of the SWI/SNF complex, and RNA polymerase II. *SLC7A11* encodes a subunit of the cystine/glutamate transporter XCT.

The regulation of the XCT transporter has recently gained considerable attention for its ability to reprogram cellular metabolism during tumorigenesis and to alter tumor cell sensitivity to anti-cancer therapies (Koppula et al., 2018). The XCT transporter facilitates the internalization of cystine into cells, which is then metabolized into cysteine that are essential for GSH synthesis (Harris et al., 2015). Indeed, cysteine levels were reduced by more than 2-fold in ARID1A-deficient cells compared to ARID1A-proficient cells, confirming a link between impaired cystine import due to SLC7A11 downregulation and decreased intracellular cysteine levels (Ogiwara et al., 2019).

Harris et al. previously showed that cysteine depletion renders breast cancer cells dependent on thioredoxin as an alternative antioxidant pathway (Harris et al., 2015). The compounds used in Ogiwara et al. study, PRIMA-1 and its analog, are known to also inhibit thioredoxin reductase and to decrease thioredoxin (TrxR) levels. Although APR-246 seemed to inhibit GSH more efficiently than TrxR, Ogiwara et al. found that ARID1A-deficient cancer cells were highly sensitive to auranofin, a TrxR inhibitor (Ogiwara et al., 2019). This result established that combined inhibition of GSH and TrxR has a synergistic inhibitory effect on ARID1A-deficient tumor cells.

Given the above, what are the molecular consequences of GSH (and TrxR) depletion in ARID1A-deficient cancer cells? Ogiwara et al. identified Noxa as a major apoptotic factor whose expression was triggered by either ARID1A deficiency or APR-246 treatment (Ogiwara et al., 2019). Noxa is a Bcl-2 homology 3 (BH3)-only member of the Bcl-2 family proteins and a key mediator of p53-induced apoptosis. Noxa has been previously shown to localize to the mitochondria where it interacts with anti-apoptotic Bcl-2 family members and triggers the activation of caspase-9, ultimately leading to apoptosis (Oda et al., 2000). Controversially, Ogiwara et al. report that, in their models, apoptosis involves p53 activation and Noxa expression is instead controlled by the JNK pathway (Ogiwara et al., 2019). However, neither JNK inhibition nor Noxa depletion affected levels of GSH or reactive oxygen species (ROS) in ARID1A-deficient tumor cells. This result suggests that the observed JNK pathway activation may be a downstream or secondary effect of high ROS levels. Therefore, the exact mechanism by which GSH depletion causes cell death in the absence of ARID1A is still unclear.

Nonetheless, the study by Ogiwara et al. has identified a role for ARID1A in redox homeostasis that has important therapeutic implications. Their findings also put ARID1A in the realm of NRF2, the master transcription factor that governs the expression of both GCLM and the glutamate cysteine ligase catalytic subunit (GCLC), and thereby controls GSH synthesis (Gorrini et al., 2013b). Indeed, Ogiwara et al. have made two
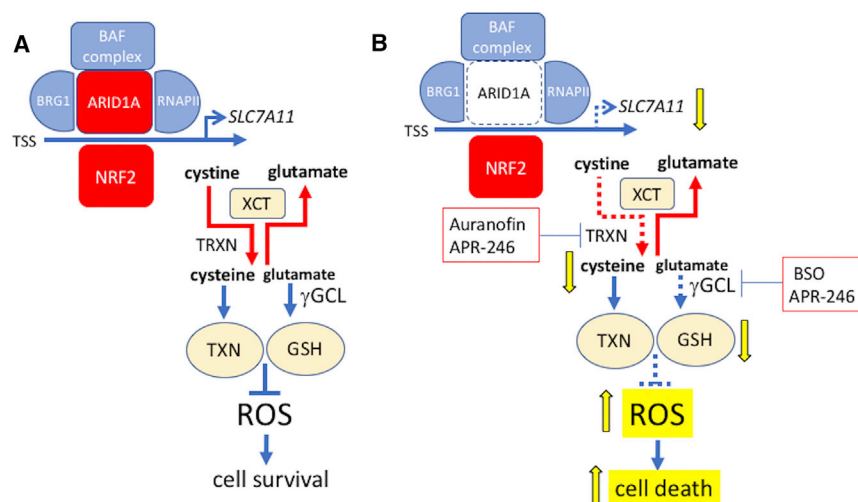
Figure 1. ARID1A Influences the Transcription of *SLC7A11* to Promote the Synthesis of Reduced Glutathione

(A) In ARID1A-proficient cells, ARID1A partners with the BAF complex, BRG1, the catalytic subunit of the SWI/SNF chromatin remodeling complex, RNA polymerase II, and the antioxidant transcription factor NRF2 to form a complex that binds to the transcription start site (TSS) of *SLC7A11*. *SLC7A11* encodes a subunit of the cystine/glutamate transporter XCT. XCT imports cystine into the cell in exchange for glutamate. The imported cystine is immediately reduced to cysteine by thioredoxin reductase (TRXN) to produce thioredoxin (TXN). Cysteine and glutamate are also utilized by γ-glutamate cysteine ligase (GCL) to produce reduced glutathione (GSH). Both TXN and GSH function to control ROS levels and prevent cell death.

(B) In ARID1A-deficient cells, *SLC7A11* is poorly expressed and XCT levels are low, depleting intracellular cystine and thus cysteine and GSH (dashed lines). Such cells are vulnerable to TRXN and GSH inhibition by auranofin, APR-246, or buthionine sulphoximine (BSO), which are compounds that further deprive ARID1A-deficient cells of antioxidant power. ROS then accumulate to unbearable levels, triggering cell death.

important discoveries: (1) ARID1A and NRF2 co-exist at the TSS of *SLC7A11*, and (2) forced NRF2 expression can restore SLC7A11 mRNA and protein levels in ARID1A-deficient cancer cells (Ogiwara et al., 2019). Thus, although ARID1A does not affect NRF2 expression per se, it contributes to the optimal regulation of SLC7A11 expression. Curiously, this is not the first time that ARID1A has been implicated in the maintenance of ROS levels. In a previous study conducted in liver cancer, Sun and colleagues showed that ARID1A can exhibit both oncogenic and tumor-suppressive functions depending on the stage of tumorigenesis (Sun et al., 2017). During the initiation phase, ARID1A promotes transformation by increasing the transcription of genes encoding cytochrome P450 enzymes (CYP450), a superfamily of monooxygenases that oxidize exogenous and endogenous metabolites and promote oxidative stress. Once a liver cancer is fully established, ARID1A loss accelerates tumorigenesis by triggering

the expression of metastasis-supporting genes. It should be noted that the scenario described in this study is quite complex and may be specific to this particular tissue context. CYP450 are major enzymes involved in xenobiotic metabolism. These enzymes are primarily found in the liver and account for about 75% of total metabolism in this organ. Expression of CYP450 genes is mainly controlled by the transcription factor aryl hydrocarbon receptor (AhR), which, together with NRF2, are the central regulators of intracellular antioxidant responses. Intriguingly, ARID1A is important for the activation of AhR expression in innate lymphoid cells (Xia et al., 2017).

In conclusion, the work of Ogiwara and colleagues has opened up a new perspective on the mode of action of epigenetic regulators during tumorigenesis. Their results clearly demonstrate that the GSH metabolic pathway is the main hub supporting the survival of ARID1A-deficient cancer cells (Figure 1). Although ROS can promote either

cell signaling or cell death by provoking cellular damage, there is a general consensus that GSH metabolism is important for tumor initiation and progression.

In light of the above, it is surprising that loss of ARID1A, which impairs cysteine and GSH levels, facilitates tumorigenesis. It may be that, just as loss of BRCA1 relieves tumor suppression but impairs NRF2 stability (Gorrini et al., 2013a), an ARID1A-deficient cancer cell has to pay the price of losing antioxidant power to maintain other tumor-promoting pathways. However, this compromise eventually proves too costly since it creates dependencies that turn into vulnerabilities. This study by Ogiwara et al. thus emphasizes the importance of adequate GSH metabolism in tumor initiation, progression, and drug resistance. A note of caution: although this newly discovered link between epigenetics and GSH may have great therapeutic relevance, these two pathways are very complex, and each has multiple functions. A better understanding of this relationship will be necessary to develop properly tailored GSH inhibitors that can be used in combination with drugs targeting epigenetic regulators to effectively limit tumor growth.

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Paradoxical Puma Prohibits Pyruvate Pumps to Prime Pathology

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PUMA is a pro-apoptotic Bcl-2 family protein that can act as a tumor suppressor or oncogene in different cancers. In this issue, Kim et al. show that PUMA, independent of its apoptotic function, enforces glycolytic metabolism by inhibiting the transport of pyruvate into the mitochondria, promoting hepatocellular carcinoma.

> Peter Piper picked a peck of pickled peppers.—Anonymous

To paraphrase the late Stan Korsmeyer, if the control of apoptosis is the "night job" of the Bcl-2 family proteins, what are their "day jobs"? The Bcl-2 family is largely defined by this night job of apoptotic regulation and the presence of one or more of the four Bcl-2 Homology (BH) regions. The largest subfamily is made up of pro-apoptotic proteins that carry only a BH3 region and hence are called BH3-only proteins. The BH3 region, itself, is generally sufficient to carry out the night job function of BH3-only proteins, and therefore, we can wonder whether these molecules have additional functions (day jobs). Prominent among the BH3-only proteins is p53-upregulated mediator of apoptosis (PUMA), and a study in this issue (Kim et al., 2019) sheds light on a novel day job for PUMA in regulating metabolism in hepatocytes and hepatocellular carcinomas.

As its name implies, PUMA is induced by p53 (and also by p73), and it is required for DNA damage-induced apoptosis in thymocytes, fibroblasts, and hematopoietic stem cells. Its expression is also induced in p53-independent ways, such as by Foxo3A upon growth factor deprivation, and C/EBP homologous protein and E2F1 following endoplasmic reticulum stress, and upon overexpression of c-Myc (Hikisz and Kiliańska, 2012). Once expressed, the BH3 of PUMA binds to the anti-apoptotic proteins Bcl-2, Bcl-xL, Mcl-1, and A1/Bfl, neutralizing them, and it can also directly activate the pro-apoptotic effectors Bax and Bak to cause mitochondrial outer membrane permeabilization (MOMP) and apoptosis (Green, 2018). In addition to the BH3, PUMA possesses a mitochondrial localizing sequence that directs it to the mitochondrial outer membrane, facilitating its pro-apoptotic effects.

Given these pro-apoptotic functions, it is probably not surprising that the PUMA gene is often deleted (e.g., in head and neck cancers) or silenced (e.g., in B cell lymphomas), although mutations in the coding region of PUMA have not been observed in cancers. In contrast, robust PUMA protein expression is often observed in some cancers, including colorectal carcinoma (CRC) and hepatocellular carcinoma (HCC). While deletion of the PUMA gene accelerated Myc-induced B cell lymphomagenesis,

ablation of PUMA actually prevented radiation-induced thymomagenesis and chemically induced HCC in mice (Labi et al., 2010; Michalak et al., 2010; Qiu et al., 2011). These observations present the "PUMA paradox" in cancer: why is a pro-apoptotic protein and bona fide tumor suppressor not only expressed in some cancers, but apparently even required for their generation?

Convincing attempts to explain this paradox suggest that apoptosis, such as that induced by DNA damage-induced p53 and PUMA expression, is important in driving compensatory proliferation of precursor cells, thus expanding mutant clones that become transformed upon acquisition of subsequent mutations. This idea is supported by the observation that p53 functions to suppress radiation-induced thymomagenesis, not at the initial, apoptotic phase but later as cells transform (Christophorou et al., 2006). Indeed, induction of thymocyte apoptosis by glucocorticoids promoted radiation-induced thymomas in PUMA-deficient animals (Michalak et al., 2010). It is logical to conclude that similar apoptosis and compensatory proliferation functions in chemically induced HCC (Qiu et al., 2011).