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EDITORIAL – COLORECTAL CANCER

Biomarkers of Bad Biology: Curse or a Blessing?

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Epigenetic regulation of the cysteine dioxygenase type 1 (CDO1) gene is a frequent alteration across a wide variety of cancer types, including common (i.e., colorectal, prostate, breast cancers) and rare (i.e., gallbladder and esophagus) malignancies.[1–5] In these diseases, hypermethylation of the *CDO1* promoter results in decreased expression of CDO1 enzyme and reduced conversion of cysteine to cysteine sulfinic acid (CSA). Several studies have found that *CDO1* promoter hypermethylation, and resultant reduction of *CDO1* expression, directly correlates with disease progression, suggesting that CDO1 has a tumor suppressive effect. In turn, *CDO1* promoter hypermethylation is associated with poor prognosis.[1,6] Contrary to these reports, the current study, "Epigenetic status of *CDO1* gene may reflect chemosensitivity in colon cancer with postoperative adjuvant chemotherapy" by Yokoi et al., describe a paradoxical finding that high levels of *CDO1* promoter hypermethylation in patients with stage III colorectal cancer (CRC) have a better prognosis following resection.[7] This difference in outcome does not persist in subset analyses of patients who did not receive adjuvant chemotherapy. In fact, only patients with high *CDO1* promoter hypermethylation who received adjuvant oxaliplatin-based therapy had better overall survival. Through this careful, albeit retrospective analysis, the authors uncover a situation where the natural history of disease is reversed by systemic chemotherapy in patients with "bad" tumor biology.

Normally, CDO1 functions to synthesize CSA, which shunts cysteine away from the production of gluthionine (GSH), a potent reactive oxygen species (ROS) scavenger.[8] In turn, low GSH promotes cellular sensitivity to ROS-mediated inflammation and apoptotic cell death. In line with this cascade, reduction of CDO1 levels leads to increased GSH, thereby promoting cancer cell survival.[9] Contrastingly, Prabhu et al. reported that reduction of CDO1 expression (and CSA concentration) decreased glioblastoma multiforme (GBM) tumor development.[10] These results support the concept that CDO1 may also have oncogenic properties in certain cancer types. In the current study, Yokoi et al. corroborate the latter findings in that forced overexpression of CDO1 results in improved cell viability in an anaerobic environment and increased chemoresistance, supporting a pro-survival role of CDO1. The clinical observation that high levels of *CDO1* promoter hypermethylation are associated with prolonged survival following administration of adjuvant chemotherapy suggests that *CDO1* epigenetic regulation may be a biomarker of drug response. However, this is inferred and not directly proven in this study. Moreover, the function of *CDO1* as a tumor suppressor gene is not quite so simple.[1] The terms oncogene and tumor suppressor gene serve as intuitive and convenient models for genetic alterations that can give rise to unregulated cell growth. However, this classification system often ignores the temporal and spatial regulation of genes, transcripts, and proteins that can result in mixed oncogenic and tumor suppressive functions. There is no better example of this than TGF-β, which serves as a tumor suppressor in epithelial cells, but often is hijacked in advanced carcinomas to promote tumor metastasis.[11] While CDO1 has a tumor suppressor function related to cysteine homeostasis, the present study and others suggest that CDO1 expression is associated with hypoxic cell

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viability, chemoresistance, and metastasis. Clearly, the function of CDO1 is more complex than the simple dichotomy of oncogene versus tumor-suppressor gene.

Another key in vitro result in the present study is that expression of *CDO1* elevates mitochondrial membrane potential (MMP), which directly or indirectly determines susceptibility to chemotherapy. In turn, overexpression of *CDO1* rendered cells resistant to the nucleoside analog, 5-fluorouracil (5-FU). This finding may provide a hypothesis for the clinical observation that patients with high promoter hypermethylation (i.e., suspected low CDO1 expression and decreased MMP) had longer median overall survival (i.e., due to a suspected increase in chemosensitivity) compared with patients with low promoter hypermethylation. Interestingly, this finding somewhat contradicts the observation that silencing of *CDO1*, which promotes ROS detoxification, contributes to survival of breast cancer cells following anthracycline-induced oxidative stress.[9] This may be due to the differences in the mechanism of cytotoxicity between anthracyclines and an oxaliplatin-based regimen. In the current study, it is intriguing that chemotherapy treatment in patients with high *CDO1* promoter hypermethylated tumors (i.e., a biomarker for "bad biology") actually had better survival outcomes.

There are several examples of similar scenarios in cancer therapy. Historically, mutations in *ERBB2* were associated with dismal outcomes in breast cancer patients. However, this has changed since the introduction of trastuzumab, a monoclonal antibody targeting the *ERBB2* gene product, HER2/Neu. Now, in the era of trastuzumab treatment, long-term outcomes are roughly equivalent amongst patients with HER2/Neu-positive or -negative breast cancer.[12] Another landmark discovery was the identification of low methylguanine-DNA methyltransferase (MGMT) expression, often due to *MGMT* promoter hypermethylation, as a biomarker of sensitivity to alkylating agents, such as temozolomide and dacarbazine, in patients with high-grade gliomas.[13,14] Mechanistically, alkylating agents damage DNA by promoting formation of cross-linked double stranded DNA. Although these lesions are typically repaired by MGMT, patients with reduced expression of MGMT remain sensitive to the effects of DNA alkylating agents. A similar approach is being investigated in gastrointestinal stromal tumors (GIST), paragangliomas, and pheochromocytomas arising from succinate dehydrogenase complex (SDH) deficiency.[15] These tumors have global DNA hypermethylation, and therefore, there is an ongoing Phase II clinical trial (NCT03165721) investigating treatment with a DNA methyltransferase inhibitor, guadecitabine, that may reverse DNA hypermethylation in tumor cells.[16] Finally, another recent example of pharmacogenomics utilizes the concept of synthetic lethality, or when the combination of two gene deficiencies causes cytotoxicity, whereas individual deficiencies are insufficient to cause cell death. Germline mutations in the *BRCA1/2* genes are associated with hereditary forms of breast, ovarian, prostate, and pancreas cancer. Under normal circumstances, the BRCA complex functions in homologous recombination repair of double-stranded DNA breaks while the distinct poly(ADP-ribose) polymerase (PARP) family of proteins function as DNA damage sensors. Treatment of BRCA-associated tumors with PARP inhibitors has been shown to promote the formation of double stranded DNA breaks that leads to synthetic lethality in tumor cells.[17,18] The current findings in CRC further highlight the importance of molecular-guided risk stratification and patient selection, even for traditional systemic chemotherapeutic regimens. Now, in the burgeoning era of personalized and precision cancer treatments, traditional systemic chemotherapies also must be guided by emerging molecular biomarkers.

According to National Comprehensive Cancer Network (NCCN) and European Society for Medical Oncology (ESMO) guidelines, the current paradigm for treating patients with stage III CRC entails adjuvant chemotherapy.[19,20] Presumably, this is due to elimination of micrometastatic disease present at the time of resection. Several large, clinical trials have validated the survival benefit for treating this group of patients.[21,22] In contrast, adjuvant therapy for stage II CRC is generally not recommended according to these guidelines, because subset analyses from large clinical trials (e.g., IMPACT B2 Analysis, 1999; Intergroup Analysis, 2004; Cancer Care Ontario Program, 2004; QUASAR, 2007; MOSAIC, 2009; NSABP C-07, 2007) have failed to demonstrate a benefit of systemic therapy in this patient population.[23] However, there are high-risk features that may warrant consideration of adjuvant treatment for stage II disease. These markers can be classified into clinical variables and molecular biomarkers. The well-accepted clinical factors associated with aggressive disease include pathologic T4 stage, tumor perforation, lymphovascular invasion, perineural invasion, and inadequate lymph node sampling.[23] There also are several molecular biomarkers that have been shown to be associated with high-risk stage II disease, including microsatellite instability (MSI), *KRAS* mutations, *BRAF* mutations, and low *CDX2* mRNA expression.[23,24] The findings now presented in the study by Yokoi et al. raise the interesting possibility that *CDO1* promoter hypermethylation may also have a prognostic role in stage II disease. Retrospective query into the efficacy of adjuvant chemotherapy in patients with stage II CRC and high *CDO1* promoter hypermethylation may be useful in determining the applicability of this biomarker. It is possible that patient selection for clinical trials based on *CDO1*

promoter hypermethylation may better select subjects with "bad biology" but also the "blessing" of potential benefit from adjuvant therapy.

In the era of personalized-precision medicine, uninformed treatment of cancer is rapidly losing relevance.[25] While focused molecular testing is an increasingly common practice for matching genomic alterations with cognate targeted agents, the findings presented here underscore the importance of epigenetic analyses in tailoring cancer treatment.

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