Targeting the aryl hydrocarbon receptor/polyamine biosynthesis axis of evil for cancer therapy

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Commentary

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Polyamine metabolism as a target for anticancer therapy

Polyamine metabolism is frequently dysregulated in cancer (1) and, as such, represents a rational target for anticancer therapy. Several preclinical studies suggested that inhibition of the enzyme ornithine decarboxylase (ODC), encoded by the ODC1 gene, by the enzyme-activated irreversible inhibitor D,L-α-difluoromethylornithine (DFMO) would be an effective clinical agent. Unfortunately, although DFMO was well tolerated, it was not effective as a single agent in the treatment of established cancers in the clinic (2). In the accompanying article by Bianchi-Smiraglia et al., strong evidence is provided for an alternative method of reducing ODC activity, and reducing polyamines in malignancies, by inhibiting a newly discovered transcriptional activator of ODC1, the aryl hydrocarbon receptor (AHR) (3).

Prior to fully appreciating the significance of their work, one must get into the weeds of polyamine metabolism. Although a rational drug target for cancer therapy, polyamine metabolism is somewhat unique (4). ODC, like many enzymes, is regulated at the transcriptional, translational, and posttranslational levels. However, unlike other enzymes, it is also regulated by other proteins that directly affect its stability. It is this regulatory dance that the current work proposes to disrupt.

After transcription/translation of the ODC1 gene in response to growth stimuli, the ODC protein must form a homodimer for activity (5). When polyamine levels are below the cellular set point, homodimer formation is favored. However, a second protein known as ODC antizyme (AZ) has high affinity for monomeric ODC (6, 7). When present, AZ binds to the ODC monomer, blocks dimerization, inhibits ODC activity, and ultimately chaperones ODC monomers to the 26s proteasome for ubiquitin-independent degradation. As if that isn’t complicated enough, nature has devised another protein for ODC regulation: an inactive ODC homolog known as ODC antizyme inhibitor (AZIN1), produced when polyamine concentrations are low. AZIN1 has a higher affinity for AZ than does ODC; consequently, AZIN1 can bind to AZ, block its inactivation/degradation of ODC, and increase the polyamine biosynthetic capacity of the cell.

Both ODC and AZIN1 have been implicated as oncogenes (8–10). Consequently, pathways leading to increased expression of these two proteins have the potential to lead to loss of growth control and carcinogenesis.

Identifying AHR as a transcriptional regulator of polyamine biosynthesis

Bianchi-Smiraglia et al. identified a new signaling pathway that activates the transcription of both ODC1 and AZIN1. Using bioinformatics, they identified AHR binding sites in the promoters of both genes. They then demonstrated that AHR directly activates the transcription of both ODC1 and AZIN1, with the ectopic expression of a constitutively active form of AHR and by ChIP analysis in WI-38 human fibroblasts. Luciferase constructs confirmed an active transcriptional role of AHR in both genes in response to a cotransfected, activated AHR construct or treatment with benzo[a]pyrene, a known activating ligand of AHR. Next, since ODC1 is a known transcriptional target of MYC and because MYC itself has been implicated as a target of AHR, they went on to show that mutating the MYC-binding element in the ODC1 promoter had no effect on AHR-mediated transcription. Finally, to confirm that AHR was directly involved in ODC1 and AZIN1 expression, they either used siRNA or a known AHR antagonist, CH223191, to reduce AHR levels. In both cases, the expression levels of ODC1 and AZIN1 decreased and were accompanied by a decrease in polyamines.

These results are provocative for multiple reasons: (a) They suggest that AHR interference could be an effective targeting strategy to reduce ODC1 and AZIN1 expression; (b) they indicate that if there are neoplasms that rely on this signaling pathway for the increase in polyamines
necessary for tumor proliferation, AHR signaling may be a potential target for anticancer therapy. Importantly, although AHR is postulated here to only affect the biosynthetic pathway, specifically, the production of the polyamine putrescine, the data indicate a rapid and significant depletion of all three polyamines when AHR is inhibited. If only polyamine biosynthesis were inhibited, the loss of the higher polyamines would only occur through dilution by division. However, the rapid polyamine depletion suggests that inhibiting AHR also has a role in inducing polyamine catabolism (Figure 1, and see below) (11).

**Identifying an effective drug targeting AHR to reduce polyamine biosynthesis**

Since their data are consistent with the hypothesis that AHR signaling is a rational drug target, Bianchi-Smiraglia et al. went on to identify small-molecule AHR antagonists useful in targeting polyamine metabolism. Only one drug, clofazimine (CLF), demonstrated a dose-dependent decrease in ODC1 and AZIN1 within 2 hours of treatment in WI-38 fibroblasts, similar to the effects of CH223191. Since CLF is a clinically approved drug for the treatment of leprosy, the authors extended their studies to confirm the mechanism of action of CLF and its potential utility as an antineoplastic agent.

Importantly, CLF treatment decreased all polyamines, again suggesting inhibition of polyamine biosynthesis and induction of the polyamine catabolic pathway. Thus, in addition to showing that ODC expression was down, the authors also provide evidence that the two important enzymes in polyamine catabolism, spermidine/spermine N1-acetyltransferase (SAT1) and spermine oxidase (SMOX) are increased, thus explaining the rapid decrease in polyamines. Interestingly, SMOX has multiple putative AHR binding sites in its promoter region, suggesting that its transcription may also be regulated by AHR. However, in the case of SMOX, AHR would have to be a transcriptional repressor to respond to CLF with increased expression (https://www.encodeproject.org/).

The role of AHR in cellular pathology is complicated; it has been described as both a tumor suppressor and an oncogene (12, 13). To determine the subset of cancers that might benefit from targeting AHR, the authors surveyed 26 different cancer data sets, identified three specific tumor types whose expression of AHR was inversely correlated with survival, and chose multiple myeloma as their model. To confirm the link between AHR expression and polyamine metabolism in myeloma patients, they devised a polyamine synthesis score based on gene expression data from a patient data set. Patients with the highest expression of AHR and high-
est polyamine biosynthesis score had the poorest survival.

To further confirm the link between AHR, polyamines, and myeloma, three different human myeloma cell lines, MM.1S, RPMI-8226, and U266, were used to recapitulate their findings in the WI-38 cells. Significantly, since U266 does not express AHR, it served as a negative control. While pharmacologic or shRNA-mediated inhibition of AHR signaling was effective in reducing cell growth and polyamine biosynthesis in the MM.1S and RPMI-8226 cell lines, it had little effect in U266 cells. Further, addition of spermidine to CLF-treated cells partially rescued them from the growth-inhibitory effects of the AHR antagonist. There also was no evidence that MYC played a role in the response to AHR signaling.

**CLF is effective in treating bortezomib-resistant multiple myelomas**

Bortezomib (BTZ) is a front-line therapy for multiple myeloma; however, relapse with BTZ resistance occurs frequently (14). To determine whether BTZ-resistant cell lines were susceptible to CLF, the authors generated BTZ-resistant clones of MM.1S and RPMI-8226. The results of these studies indicate that BTZ-resistant myeloma cells maintain their sensitivity to CLF. Unfortunately, combination studies of BTZ-resistant and -sensitive cells were not reported here.

Finally, CLF activity was evaluated in xenograft mouse models of MM.1S and RPMI-8226. The in vivo results confirmed the efficacy of CLF in the treatment of these models and demonstrated similar efficacy for BTZ. Additional confirmation of CLF activity was demonstrated in a VkMYC mouse model that spontaneously develops multiple myeloma. Here, too, CLF significantly reduced disease burden with a response comparable to that elicited by BTZ.

**Conclusions**

The results of these studies are very encouraging in that they identify an entirely new target and drug for malignancies such as multiple myeloma. The high expression of AHR and the dependence on polyamines for growth and survival in myeloma suggest a promising way forward in treating difficult diseases that express high levels of AHR. What would also be very interesting to know is the effect of drug combinations on these tumor models. The combination of BTZ and CLF should certainly be considered. Even more important would be the combination of CLF and DFMO. DFMO is clinically approved for the treatment of infection with a specific species of trypanosome, the parasite responsible for African sleeping sickness (15). DFMO is extremely well tolerated and is actively being studied in clinical trials as a chemopreventive agent in gastrointestinal and other cancers (16, 17) and in combination with other agents in the treatment of neuroblastoma and other malignancies (18–20). The idea of targeting ODC expression and activity at multiple steps, from transcription to stability to activity, is attractive, because the development of resistance to all mechanisms occurring at once is unlikely. Finally, it would be interesting to determine whether induction of polyamine catabolism is essential for the antitumor effect of CLF, as has been indicated for several antitumor polyamine analogs (21).

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