Say What? The Activity of the Polyamine Biosynthesis Inhibitor Difluoromethylornithine in Chemoprevention Is a Result of Reduced Thymidine Pools?

Robert A. Casero Jr

Summary: In this issue of *Cancer Discovery*, Witherspoon and colleagues use an unbiased metabolite profiling approach to study the effects of polyamine depletion by 2-difluoromethylornithine in colon cancer cells. Their surprising findings indicate that it is a decrease in thymidine pools resulting from altered tetrahydrofolate availability rather than decreases in polyamines that produces cytostasis. *Cancer Discov;* 3(9); 975–7. ©2013 AACR.

See related article by Witherspoon et al., p. 1072 (7).

Targeting polyamine metabolism and function as a strategy for cancer chemotherapy and chemoprevention remains a rational and promising approach in multiple solid tumor types (1, 2). However, because the underlying molecular mechanisms of successfully exploiting this strategy have been slow to emerge and because of the initial disappointing results in early clinical trials when polyamine metabolism was targeted with single agents, some in the field have lost enthusiasm for pursuing this important target in the search for successful treatment of neoplastic disease. That 2-difluoromethylornithine (DFMO), an enzyme-activated, irreversible inhibitor of the first rate-limiting step in polyamine biosynthesis, can be used for the successful and safe treatment of human disease is not in question, as it has been successfully used alone and in combination in the treatment of African sleeping sickness caused by the parasite *Trypanosoma brucei gambiense* (3, 4). Moreover, there has been a resurgent interest in DFMO as a chemopreventive agent, particularly in colon cancer, based largely on the results of Meyskens and colleagues (5). Although clinical trials combining DFMO with nonsteroidal anti-inflammatory (NSAID) agents have been encouraging, the precise molecular mechanisms underlying the DFMO effect are not clear, as the clinical efficacy does not appear to correlate with changes in epithelial polyamine concentrations (6).

In this issue, Witherspoon and colleagues (7) present data supporting the intriguing hypothesis that inhibition of polyamine metabolism by DFMO leads to perturbations in single carbon reactions through the increased consumption and regeneration of S-adenosylmethionine (SAM), resulting in decreased availability of tetrahydrofolate (THF) for the synthesis of thymidine (Fig. 1). Using the results of an elegant unbiased metabolite profiling strategy, they put forward a convincing argument that under their experimental conditions, it is the decrease in thymidine and not the loss of polyamines that is responsible for the growth-inhibitory effects of DFMO treatment. This hypothesis is supported by the fact that near-normal growth can be restored by the classical add-back of polyamines to DFMO-treated cells, but they also show that the supplementation of thymidine prevents DFMO-induced cytostasis without restoring intracellular polyamine pools.

That polyamine depletion and subsequent changes in SAM pools could produce significant changes in single-carbon reactions, including nucleic acid and protein methylation, is not a new idea. Papazafi ri and Osborne (8) showed significant decreases in DNA methylation after polyamine depletion by DFMO, which they attributed to decreases in SAM and increases in decarboxylated SAM. Yarlett and Bacchi (9) showed in *Trypanosoma spp.* that DFMO treatment affected methionine cycle intermediates. Yamamoto and colleagues (10) showed that inhibition of ornithine decarboxylase (ODC) by ODC antizyme resulted in increased decarboxylated SAM that led to decreases in both DNA methylation and the methylation of lysine 9 of histone 3. ODC antizyme is an ODC regulatory protein that binds to, inhibits, and then facilitates the proteosomal degradation of ODC, thus having some of the same effects as ODC inhibitors (11).

More relevant to the current study, Bistulfi and colleagues (12) showed that high flux through the polyamine metabolic pathway resulting from increased polyamine catabolism by spermidine/spermine N1-acetyltransferase (SSAT), a rate-limiting step in polyamine catabolism, placed high demand on the SAM pools and had a significant impact on sensitivity to folate depletion. Specifically, they found that the greater the flux through the polyamine metabolic pathway, the greater the requirement for folate. Importantly, when polyamine biosynthesis was blocked by a specific inhibitor of SAM decarboxylase, the cellular demand for folate was significantly reduced. The results of each of these previous studies are completely comprehensive.
consistent with the findings of the current studies. However, there are some interesting contrasts with other \textit{in vitro} and \textit{in vivo} studies with DFMO.

Specifically, it is interesting to note that the current results show significant decreases in the higher polyamines with DFMO treatment, both in cell culture and \textit{in vivo}. These results are in contrast to the lack of spermine depletion that has previously been reported (13, 14). The reasons for these differences are not entirely clear, but may result from longer treatment times with a lower dose of DFMO than reported in earlier studies or differences in routes of administration. Most previous \textit{in vivo} studies administered DFMO in the drinking water, whereas Witherspoon and colleagues (7) used intraperitoneal injection. Although seemingly counterintuitive, the major mechanism for loss of spermine in the absence of increased polyamine catabolism is dilution through division. Consequently, if the lower dose of DFMO used in the current study allows more divisions until thymidine concentrations become growth limiting, such decreases in both spermidine and spermine may be explained.

Regardless of the underlying reasons for these differences, the current results support at least one alternative hypothesis to direct polyamine depletion as the molecular mechanism responsible for DFMO-induced cytostasis in a chemopreventive setting. This finding has significant implications as the development of DFMO for chemoprevention moves forward. Most immediately, it suggests the potential for SAM, folate cofactors, and thymidine levels to be used as biomarkers to identify individuals who might benefit most from DFMO therapy.

It will also be interesting to see whether follow-up unbiased metabolite studies are carried out using the combination of DFMO and a clinically relevant NSAID, as it is likely that as DFMO goes forward as a chemopreventive therapy for colon cancer, it will be used in combination with an NSAID. As

\begin{figure}
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\caption{Inhibition of polyamine biosynthesis by DFMO leads to increased S-adenosylmethionine decarboxylase (SAM-DC) activity, ultimately diminishing THF pools available for thymidine synthesis. When ornithine decarboxylase (ODC) is inhibited by DFMO, there is not only a decrease in polyamine pools, but also a rapid increase in SAM-DC that draws on THF pools to regenerate methionine and SAM. Witherspoon and colleagues (7) propose that if the shunting of THF for increased SAM synthesis continues, the decrease in THF pools results in decreased synthesis of deoxythymidine monophosphate (dTMP), which becomes limiting and induces cytostasis. It is by this mechanism that they posit that DFMO is an effective chemoprevention agent. The green arrows indicate increasing activity of the indicated enzyme or flux through the indicated pathway. Red arrows indicate decreased concentrations of the indicated polyamine or decreased availability of the THF metabolite substrate necessary for dTMP synthesis. Note that green arrows indicate an increase in spermidine/spermine N\textsuperscript{1}-acetyltransferase (SSAT) activity in response to NSAIDs, which have been commonly used in combination with DFMO in colorectal cancer chemoprevention trials. This increase in polyamine catabolism could increase the flux through the pathway regenerating SAM when ODC is inhibited and thus synergize with DFMO in reducing the availability of THF for dTMP synthesis. MTA, methythioadenosine; dc-SAM, decarboxylated SAM.}
\end{figure}
sulindac and other NSAIDs have been implicated in altering polyamine metabolism by increasing polyamine catabolism through increased SSAT activity (15), the combination of DFMO with NSAIDs may actually increase the flux through the polyamine pathway, leading to a greater demand on the SAM pools and resulting in even less THF available for thymidine synthesis.

Finally, the results of the current study implicating the depletion of thymidine as the mechanism responsible for cytostasis in cells depleted of polyamines by DFMO may require a rethinking as to how to best approach targeting polyamine metabolism for either chemoprevention or chemotherapy. It may be possible, with this new information in hand, to better exploit this central metabolic pathway for therapeutic benefit.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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