

Cell Cycle News & Views

Neighborhood matters: Response to EZH2 methyltransferase inhibitors

Comment on: Amatangelo MD, et al. *Cell Cycle* 2013; 12:2113–9;
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Enhancer of zeste homolog 2 (EZH2) has been found to be overexpressed in ovarian and other cancers and plays critical roles in cell proliferation, angiogenesis, and inhibition of apoptosis.^{1,2} Among its many functions, it tri-methylates lysine residue at the 27 position of histone H3 associated with the target gene.³ EZH2 is widely considered an important target for cancer therapy. Several approaches to block EZH2, including inhibitors (e.g., GSK126, GSK343, E1) and siRNA incorporated into nanoparticles, are being examined.^{2,4–6}

It is becoming increasingly clear that 2D in vitro assays may not fully simulate the complex tumor microenvironment, likely due to differences in gene expression, lack of histological differentiation and cell–cell or cell–ECM (extracellular matrix) interactions in 2D assays. On the other hand, tumor cells grown in 3-dimensional settings are morphologically and histologically closer to those growing in vivo.^{7,8} Some studies have shown that cells in 3D conditions are more resistant to chemotherapy and require larger doses of drug as compared with 2D culture.⁸ Therefore, many investigators have started to use 3D cultures for in vitro drug testing. In the recent issue of *Cell Cycle*, Zhang and colleagues observed that GSK343, a methyltransferase inhibitor, had minimal to no effects on ovarian cancer cells under 2D culture setting. Interestingly, there was a significant decrease in cell proliferation in 3D cultures with GSK343 treatment.

Treatment also changed the morphology of cells to normal epithelial-like phenotype and suppressed invasion in 3D cultures. To investigate downstream signaling, the authors checked the expression of a pro-apoptotic H3K27Me3 target gene, HRK (Harakiri), and found that it is significantly upregulated in 3D, but not in 2D settings. These observations suggest that the tumor microenvironment plays a very important role in regulating the efficacy of methyltransferase inhibitors.⁵

Although the authors have convincingly shown differences in drug efficacy in vitro; in vivo studies are essential to further develop these drugs. EZH2 has been shown to play diverse roles in cancer biology including angiogenesis and maintenance and expansion of cancer stem cell populations.^{1,2} Thus EZH2 is an attractive target for cancer therapy and the current study provides an important step forward related to crosstalk between EZH2 and ECM. Differential expression of certain genes in 3D conditions might be playing an important role in sensitizing the cells to GSK343. Additional knowledge about the differences in signaling molecules will not only help to understand the exact mechanism by which 3D setting alters the response of cancer cells to the inhibitors, but may also suggest some potential therapeutic targets. Additional experiments with other cell types in the tumor microenvironment, such as fibroblasts, endothelial cells and macrophages, in

a hypoxic environment to study their effects on sensitization of tumor cells could further inform mechanisms by which these inhibitors function.

Overall, the study by Zhang and colleagues provides new and important knowledge regarding the therapeutic potential of EZH2 inhibitors. Such studies coupled with additional in vivo work are essential for providing a path toward clinical development of such inhibitors. The expanding body of preclinical data regarding EZH2 strongly justifies further development of this important target.

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Sizing up circulating tumor cells for personalized therapy

Comment on: Gallant J-N, et al. *Cell Cycle* 2013; 12:2132–43;
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Most deaths resulting from solid cancers are not caused by the primary tumor but by metastases to distant organs.¹ Such metastases require that the cancer escapes the primary microenvironment and spreads via the bloodstream. The study of such blood-borne cancer cells (circulating tumor cells, CTCs) offers a unique window into the process of metastasis. Although CTCs have been postulated to exist since the 19th century, it was not until the past decade that several groups have combined to develop a wide array of technologies to capture, enumerate, and characterize these cells. Approaches that capitalize on epithelial antigens,² electromagnetic properties of cells,³ and blood flow dynamics⁴ have all been explored as means to separate CTCs from whole blood. The greatest success has been achieved with antigen-based capture: based on a set of landmark studies,^{5,6} the US Food and Drug Administration (FDA) approved the clinical use of the Veridex CellSearch System (which enumerates CTCs separated from blood based on their epithelial properties) for use in patient prognosis.

It is becoming abundantly clear that the biological and clinical value of CTCs exceeds their mere enumeration. For example, a recent study by Liu et al. demonstrated that HER2-positive breast cancer patients with HER2-positive CTCs have longer progression-free survival (PFS) after anti-HER2 therapies than HER2-positive patients with HER2-negative CTCs.⁷ Emerging evidence indicates that CTCs, much like the primary tumor, are heterogeneous in nature and may include subsets of

cells that can successfully form metastases and/or cells that may be capable of re-seeding the primary tumor. Most existing technologies do not allow for capture of live/viable cells that would enable the functional determination of such metastatic potential, aggressiveness, and/or chemotherapeutic sensitivity. Technologies that are dependent on epithelial markers are likewise ineffective or will likely be minimally effective in capturing cells that are losing epithelial properties and gaining mesenchymal properties, cells that are not of epithelial origin such as melanomas (or other solid cancers of neural crest origin), or cells that have low expression of epithelial antigens such as triple-negative breast cancers.

To overcome the limitations of other CTC enrichment methods, to capture the heterogeneity of CTCs, and to broaden the functional utility of these cells, Gallant et al. have utilized a unique approach to enrich for these rare cells based on size and deformability.⁸ Using a novel technology, the FMSA device, Gallant et al. show how CTCs can be isolated from blood, potentially cultured in vitro and in vivo and thereafter used to evaluate the sensitivity of a patient's own CTCs to anticancer therapeutics. Notably, the FMSA device (along with cancer cells isolated from blood) was implanted into a mouse and tumors grew. There is potential for patient CTCs (after isolation via the FMSA or similar devices) to similarly be implanted. Such an approach has the potential to deliver in vivo chemosensitivity information and lead to the establishment of CTC-cell lines from freshly isolated tumor cells.

By allowing for the capture and maintenance of viable patient-derived CTCs, Gallant et al.'s approach should allow for functional studies not possible with other technologies. Major hurdles remain, including the need for expansion, proliferation, and growth of live/viable patient CTCs. However, cell proliferation and growth is not a limitation for genomic and biological studies of these cells. Although studying the heterogeneity of CTCs adds another layer of complexity to our understanding of cancer, it opens up a new therapeutic possibility: personalized therapeutic targeting of blood-borne cancer cells before occult metastases.

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“Naked” FACT is unstable

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A central dogma in biology states that the flow of biological information is from DNA to RNA to proteins. This model originally predicted that any changes in the levels of mRNA would lead to concomitant changes in protein levels. However, it appeared that the relationships between the levels of mRNAs and the levels of proteins they encode, particularly in eukaryotes, are not that straightforward. In mammals, less than half of all genes reveal a positive correlation between mRNA and protein levels and the correlations of transcripts and proteins varies depending on the cellular location and biological function of the gene.¹ Clearly, in eukaryotes post-transcriptional control of gene expression plays an important role in modulating protein levels.² Eukaryotic mRNAs do not exist in cells as naked polynucleotides, but rather are represented by messenger ribonucleoprotein complexes mRNPs.³ mRNA binding proteins may affect almost every aspect of mRNP metabolism from transport to localization, translation and turnover.³ In addition, protein levels are greatly influenced by co- and post-translational modifications.⁴ While the impact of proteins on mRNA stability is well known,³ what has been unclear thus far is whether proteins can be stabilized by interactions with mRNA(s). Evidence in support of the later has been very scarce.

In the current issue of *Cell Cycle*, Safina et al. presented an unusual finding suggesting that mRNAs may stabilize proteins against degradation.⁵ This paper revealed an unprecedented interplay between expression and stability of the two subunits of the Facilitate Chromatin Transcription (FACT) chromatin remodeling complex and the mRNAs encoding the FACT two subunits.⁵ FACT is a heterodimer of the 80 kDa, so-called Structure-specific recognition protein 1 (SSRP1), and the 140 kDa, so-called

Suppressor of Ty16 (SPT16) protein. FACT complex is involved in multiple processes such as DNA replication, DNA repair and mRNA elongation.⁶ Mechanisms controlling FACT cellular levels are of fundamental interest to the field of cancer biology, since FACT has been found to be frequently upregulated in cancers, particularly in poorly differentiated aggressive tumors.⁷ Moreover, suppression of FACT expression in tumor cells has been shown to lead to tumor cell death.⁸ Therefore, FACT represents an attractive target for therapeutic intervention.⁸ Mechanisms controlling FACT cellular levels have been the focus of the study by Safina et al.⁵ This study has been triggered by an observation that SSRP1 and SPT16 protein levels decline upon induction of cellular differentiation (or senescence) and that a similar decline of both proteins can be observed upon RNAi-mediated knockdown of, quite surprisingly, either of SSRP1 or SPT16 mRNAs.⁵ These results suggested that there is a cross-talk between SSRP1 mRNA and SPT16 protein levels and vice-versa. Immunoprecipitation experiments revealed that SSRP1 and SPT16 mRNAs are present in the FACT complex and further showed that this association is specific. This observation allowed the authors to suggest that the presence of the SSRP1 and SPT16 mRNAs in FACT may play a certain role in either promoting the assembly of the complex and/or its stabilization against degradation.⁵ Through a set of elegant experiments, the authors further demonstrated that neither mRNA is required for FACT complex assembly however upon binding to the FACT the mRNAs increase the stability of FACT's protein components. In addition, binding of SSRP1 and SPT16 mRNAs to the FACT complex appeared to increase the efficiency of their translation. In the absence of FACT complex, both mRNAs

are unstable and inefficiently translated making reactivation of FACT complex unlikely. The authors put forward a model in which mRNAs and particularly SSRP1 mRNA is suggested to play a key role in FACT complex stabilization. Thus, FACT complex is stable, when mRNAs are present, but rapidly degrades, when the mRNA levels drop.⁵ These findings are unique and novel. However, further detailed understanding of the exact mechanism(s) leading to FACT stabilization upon mRNA(s) binding is required. It would be of interest to map mRNA-protein binding interfaces and delineate the exact region(s) in both mRNA and protein components of the FACT responsible for FACT stabilization. Nevertheless, this is one of the first reports revealing such an unusual role of mRNA in protein stabilization. This article adds to our understanding of the multifaceted roles of RNAs in cellular homeostasis and opens up new avenues in the study of the FACT complex.

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