Reviewer’s report

Title: Cidofovir selectivity is based on the different response of normal and cancer cells to DNA damage

Version: 1 Date: 28 March 2013

Reviewer: Tohru Kiyono

Reviewer’s report:

De Schutter et al. have examined the gene expression profiles of HPV-positive cervical cancer cell lines, SiHa and HeLa, spontaneously immortalized human keratinocyte cell line, HaCaT, and primary human keratinocytes (PHKs) following exposure to the nucleotide analogue, Cidofovir (CDV). The authors also measured the amounts of CDV metabolites and incorporation of [5-3H]-CDV into cellular DNA. From the results, the authors concluded that the selectivity of CDV for HPV-transformed cells is based on the inability of HPV-positive cells to respond to DNA damage, rather than a direct anti-HPV effect. However, due to lack of an important control and a critical misunderstanding, there is a great gap between the research question posed by the authors and the conclusion.

Major Compulsory Revisions

1. The authors would like to know the underlying molecular mechanisms for the selectivity and antitumor activity of CDV against HPV-transformed cells. What kind of selectivity were the authors trying to explain? The title of the manuscript is that CDV selectivity is based on the different response of normal and cancer cells to DNA damage. This means selectivity over normal cells, and is nothing new and the conclusion is applicable for any anti-cancer drugs inducing DNA damage. CDV selectivity for HPV-positive cancer cells over HPV-negative cancer cells or over other acyclic nucleotide phosphonates (ANPs) and DNA damaging agents should be examined.

2. HaCaT is a spontaneously immortalized cell line established from cultured normal human keratinocytes. Thus HaCaT cells are never tumor cells or malignant cells though they harbor mutations in p53 and pRb genes. However, the authors call the cells as HPV-negative transformed cells (e.g., line 363, 419) and even HPV-negative malignant or tumor cells (e.g., line 217, 239,333, 337, 394, 408, 603). Many of the interpretations and the conclusions are misled by this misunderstanding. To examine the selectivity over HPV-negative cancer cells, the authors should have chosen HPV-negative cancer cell lines, such as C33A, as a control.

3. Alternatively, to examine the selectivity over other ANPs or DNA damaging agents, the authors should include the results with at least one of these agents, as a control.

4. Alternatively, the authors might want to rewrite the whole manuscript without additional experiments though the conclusions have a limited significance.
Discretionary Revisions

5. The authors’ group has already published that CDV can induce apoptosis selectively to HPV-positive cancer cell lines as well as SV40- or AdV-transformed cells compared with PHKs (Oncol Res. 1998;10(10):523-31.; Oncol Res. 2000;12(9-10):397-408.) and inhibit their tumor growth in the Xenograft model (Cancer Lett. 2013; 329(2):137-45). In the first paper, the authors also examined IC50 of CDV in human melanomas, lung, colon, and breast carcinomas cell lines. Inactivation of p53 and pRB/p16 pathways abrogate G1 checkpoint, and inactivation of p53 also attenuates G2/M checkpoint mainly through p21 and CDC25. Since E6 and E7 proteins of high-risk HPVs inactivate p53 and pRb, respectively, it has been well established that cells expressing these oncoproteins respond differently to DNA damage. Likewise, inactivation of the p53 and pRb pathway is frequently found in many cancer cells. Many anti-cancer drugs including nucleotide analogues and alkylating agents induce DNA damage on cancer cells as well as normal cells. Specificity of these anti-cancer drugs is generally based on both intensity of DNA damage and loss of cell cycle checkpoints. The authors detected the difference in such DNA damage response between HPV-positive cancer cells and normal keratinocytes. However, it does not explain the hypothetical selectivity of CDV for HPV-positive transformed cells over HPV-negative transformed cells. If off-label use of cidofovir is more effective in the treatment of high-risk HPV-associated hyperplasia than other DNA damaging agents, it might depend on different effect of CDV on replication between cellular DNA and episomal HPV genomes. However, HPV DNAs in SiHa and HeLa cells are integrated into cellular DNA, and replicated by human DNA polymerases in the same manner as cellular DNA. CDV can only act as a DNA damaging agent. Thus anti-viral effects on HPV in SiHa and HeLa cells are logically not anticipated. In head and neck squamous cell carcinomas (HNSCC), many reports suggest that HPV-positive HNSCCs are more sensitive to irradiation and anti-cancer drugs than HPV-negative HNSCCs. Since most of HPV-positive cancers retain wild-type p53 and pRb with high expression of p16INK4a, p53 can be activated and accumulated upon DNA damage though its level is less than that induced in normal cells, while it can’t be activated in p53-null cells or cells with mutant p53. Thus it is reasonable to think that the status of p53 and pRb is responsible to the different outcome upon DNA damage. As described in Abstract, antiproliferative effects of CDV have been associated with apoptosis induction, S-phase accumulation, and increased levels of tumor suppressor proteins. However, none of these important factors was directly accessed in the current study.

Despite these fundamental weaknesses of the methods used to explain for the CDV selectivity, this reviewer appreciates the thorough pathway analyses from the microarray data set. Some of the implications and speculations could be substantiated by additional experiments with combinations of different methods.

6. Key effects of CDV should be examined in parallel by different methods.

1) Amounts of key proteins, such as E6, E7 and p53, which are not regulated by transcriptionally or not detected by microarray, should be examined by Western
blots.

2) Key biological outcomes such as cell cycle arrest and apoptosis should be examined by FACS or other methods.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests.