New insight of antiretroviral drug efavirenz as anticancer agents for breast cancer therapy

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Breast cancer is the second leading cause of death in women. New therapies have emerged in the last decade that target hormone receptors in breast cancer but despite encouraging results and survival statistics, breast cancer remains a leading cause of death, mainly due to frequent therapeutic escape or limited treatment options. Thus, it is imperative to find new therapeutic strategies to treat breast cancer. It is increasingly evident that the activation of normally silent mutagenic retroelements occurs at an early stage of breast cancer and is significantly associated with lymph node metastasis. Our recent study found that the existing antiretroviral drug, efavirenz, can be used as anticancer agents by inhibiting the activity of tumor-promoting retroelements, leading to a reduction in the rate of cancer proliferation and promoting cancer cell differentiation. Newly emerging data revealed that efavirenz modulates the expression of cellular genes and miRNAs that can restore normal cellular functions. Because it is an existing drug with known safety profiles and pharmacokinetics, this antiretroviral drug could be rapidly utilized for treatment of breast cancer than they originally intended.

Keywords: Breast cancer; antiretroviral; retroelements; gene modulation


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Retro elements, including endogenous retroviruses and retrotransposons, are endogenous mobile DNA elements that utilize reverse transcriptase and RNA intermediates to relocate within the genome [1]. Although nearly half of the human genome is made up of retro elements, only a fraction of retro elements is intact and functionally active because of mutations and truncation at their 5’ end. Among the retro elements, L1 elements are the largest class of retrotransposons, of which about 80-100 copies present in human cells are classified as highly active or retro transposition competent elements [2]. All classes of retro elements that include HERVs and L1 elements, except for Alu elements, encode their own reverse transcriptase (RT) enzyme, which is required for the reverse transcription of RNA intermediates and the integration of the resulting DNA copies into new genomic sites using a ‘copy-and-paste’ mechanism. Alu elements, which lack the RT-encoding gene, instead depend on L1-encoded RT machinery. Per se, RT is most likely to be one of the highly repeated protein-coding sequences in the genome. In normal adult physiology, RT does not play any roles in cells and tissues. Accumulating evidence suggests that the RT
activity has been associated in a range of retroelement-mediated diseases and physiological disorders. While the RT of infective retroviruses has been intensively studied, L1-encoded RT has received little attention, despite the fact that several evidences indicating its role in cancer onset and progression.

Retroelements are rarely active in noncancerous cells. We and others have shown that tumors exhibit high levels of L1 activity and genomic instability (which is a hallmark of cancer) that is not present in normal tissues. When active, L1 elements actively “jump” throughout the genome. As a consequence, L1 insertions provide a source of new insertional mutations that can activate oncogenic pathways. In fact, L1 insertions are known to occur in genes that are commonly mutated in cancer. Supporting this, several tumor-specific L1 insertions have been identified in lung, prostate and colorectal cancers. Activation of L1 elements can also lead to production of chimeric transcripts containing part of the L1 sequence and part of the flanking genomic sequences including protein-coding regions, referred to as LCTs (L1 chimeric transcripts). E.g., a known metastasis suppressor gene in breast and colon cancer is silenced by the expression of an LCT, a large RNA antisense to TFPI-2 that is located ~300 kb away from the LCT site. There is also evidence that L1 contributes to cancer onset and progression by promoting the expression of many oncogenes. For example, c-Myc, one of the well-characterized transcription factors in the process of metastasis, is activated by L1 element via alternative promoters. All these studies indicate that inhibiting the activity of L1-encoded RT could prevent the expression of tumor-promoting L1 elements.

Efavirenz (EFV) is a first generation antiretroviral drug approved by FDA in 1997 widely used to treat HIV patients in combination with protease inhibitors. Interestingly, EFV is a broad-spectrum inhibitor that targets the catalytic regions of RT enzyme and blocking its activity. In our previous studies, we found that L1 expression is common in almost all the subtypes of breast tumors and breast cancer cells, and correlates with poorer patient survival. To understand the biological functions of this expression, we inhibited the activity of L1-encoded RT enzyme using efavirenz treatments. To our surprise, we found that EFV inhibition of RT leads to not only decrease the expression of L1 RT protein but also an increase in cell differentiation as measured by distinct cell shapes and borders and a decrease in cell proliferation as measured by Ki67 and FACS analysis. Notably, these phenotypic changes are reversible upon termination of EFV treatment, confirming their dependence on RT activity. To further confirm if the response to EFV is associated with changes in gene expression, we explored microarray analysis in combination with the gene ontology. A gene expression profiling showed strong up regulation of gene clusters containing genes that are involved in the biogenesis of cell surfaces, differentiation and phenotypic changes and decreased expression of genes involved in invasiveness and cell proliferation. These gene expression signatures become more apparent when the cancer cells treated with 10–15 µM of EFV for 3 days. This dose range is less than the clinically equivalent dosage recommended for HIV patients.

The underlying mechanism of gene modulation that occurs during EFV treatment is yet not clearly understood. In the exploration process of L1 RT inhibition, we have identified two possibilities to discuss with the global colleagues. One possibility was that RT inhibition might act through reversible epigenetic changes thereby be involved in the modulation of nearby genes expression by changing the epigenetic modifications of L1 elements. Second possibility was that RT inhibition could eliminate or reduce the retro transposition of L1 and Alu elements. Several computational studies proposed that L1 might regulate the network of genes by generating small noncoding RNAs or providing the transcriptional regulatory signals previously not present in the genes thereby interfering with the transcriptional machinery of cells. Strikingly, our recent deep sequencing analysis of small RNAs found that breast cancer cells in which L1 expression was silenced exhibited greatly increased expression of a number of key miRNAs and in particular, the members of let-7 miRNAs family (known to suppress stemness and promote cellular differentiation), miR-196a, miR-30d, miR-425, miR-103 and miR-200c. It seems possible that these differential expressions of miRNAs by itself could be adequate to modulate the expression of a range of genes that can restore normal cellular function. Although at present, it is unclear if there is a cross talk between the genes/miRNAs modulation and the expression of L1 elements, there is evidence suggesting that human cancer cells, which express high levels of L1 elements, have globally reduced levels of let-7 miRNAs, compared to their adjacent normal cells. Nonetheless, our studies highlight that that the existing antiretroviral efavirenz is capable of inhibiting the activity of tumor-promoting L1 elements, reverting from undifferentiated cancer cells to more differentiated normal phenotypes. We believe that these investigations can improve cancer management by specifically inhibiting the L1 expression in breast cancer cells, especially triple-negative breast cancer. Because it is an existing drug with known pharmacokinetics and safety profiles and is currently in use, EFV can be rapidly evaluated in clinical trials to evaluate its effect on breast cancer metastasis and recurrence.

**Conflicting interests**
The authors have declared that no competing interests exist.

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