The Synergistic Effect of SFK Inhibitors, Bosutinib or Dasatinib, Combined with ATRA on NB4 Leukemic Cell Differentiation was More Effective Than Combined ATRA and ATO

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Background: The acute promyelocytic leukemia (APL) has been treated with all-trans-retinoic acid (ATRA) and arsenic trioxide (ATO) for decades. Emerging evidence has implicated Src family kinase (SFK) as regulator of proliferation and survival of myeloid lineage cells. Our group studies showed that inhibition of SFK resulted enhancement of retinoic acid (RA)-induced myeloid differentiation. Recently, we founded the efficacy of the SFK inhibitor bosutinib and dasatinib on RA-induced differentiation. In this study, we investigated that SFK inhibitor enhances RA-induced differentiation with ATRA, comparing with ATRA and ATO.

Materials and Method: To determine the ability of SFK inhibition on RA-induced differentiation, NB4 cells processed in different settings were evaluated with Western blotting using a phospho-Src Y418 antibody. NB4 APL cells were treated for 72 hours as following settings: untreated control, ATRA 5 nM alone, ATO 0.5 µM alone, PP2 10 µM alone, bosutinib 0.5 µM alone, dasatinib 0.5 µM alone, ATRA plus ATO, ATRA plus PP2, ATRA plus bosutinib, and ATRA plus dasatinib. We evaluated enhancement of RA-induced differentiation by flow cytometric analysis of cell surface marker CD11b expression, granulocyte differentiation by nitroblue tetrazolium (NBT) reduction assay, and apoptosis by Annexin-V staining. In addition, we investigated to the changes in the expression of retinoic acid receptor (RAR) gene expression.

Results: SFK inhibitor PP2, bosutinib, or dasatinib induced significant enhancement of RA-induced differentiation of NB4 cells combined with ATRA. Treatment of NB4 cells with ATRA 5 nM, ATO 0.5 µM, PP2 10 µM, bosutinib 0.5 µM, or dasatinib 0.5 µM alone for 72 hours resulted in only 9.3%, 5.1%, 4.4%, 9.3%, or 7.2% of CD11b-positive cells, respectively. Co-treatment with ATRA plus ATO resulted in enhancement of CD11b-positive cells (17.4%) and co-treatment with ATRA plus PP2, ATRA plus bosutinib, or ATRA plus dasatinib resulted in 49.5%, 31.4%, or 53.2% of CD11b-positive cells, respectively. SFK inhibitors combined with ATRA enhanced more dramatic enhancement RA-induced differentiation than ATRA plus ATO (p<0.05). These results were confirmed in morphologic analysis by NBT staining, whereas these effects were not related to apoptosis. The expression of proteins derived from RAR gene expression also markedly increased in cells treated with ATRA plus SFK inhibitors than ATRA plus ATO.

Conclusion: Our data showed that bosutinib and dasatinib enhanced RA-induced myeloid differentiation when combine with ATRA. The synergistic effect of SFK inhibitors and ATRA on NB4 cell differentiation was more effective than combined ATRA and ATO. These findings suggest the combination of FDA approved SFK inhibitors, such as bosutinib and dasatinib, may be beneficial for the treatment of APL with a combination of ATRA.

Keywords: Src-Family kinases, Acute promyelocytic leukemia, All-Trans-Retinoic acid, Arsenic trioxide, Bosutinib, Dasatinib