Role of microRNAs in the activity of new therapeutic inhibitors of Src kinases in CD133+ colorectal cancer cells

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Colorectal cancer (CRC) is the second most common cause of cancer-related death (after lung cancer) and, recently CRC stem cells have been identified as CD133+ CRC cells and isolated. In CRC cells, a significant increase of cellular Src kinase activity over that observed in the adjacent normal mucosa, with its activation being linked to malignant potential has been observed. Based on this background, our hypothesis is that a strong inhibition of Src kinase in CD133+ CRC cells can be beneficial for the therapy of this type of cancer. To this purpose new inhibitors of Src phosphorilation were utilized in order to study and inhibit the progression of CRC at the level of CD133+ CRC cells.

Decrease of HT-29 colon cancer cell number after treatment with different novel Src inhibitors.

The novel Src kinase inhibitors SI34 - SI83 - S7 - S13, from pyrazol [3,4,d-] pyrimidine derivatives, have been tested on the CD133+ in HT-29 cell line (about 98% CD133+ cells). The dose response curves have been performed by using the concentrations of 2 - 10 - 25 - 50 μM and measuring the effect in terms of total cell number after 24 and 48 hours of treatment. Whereas SI34 e SI83 are effective at concentrations of 25 μM after 48 hours of treatment, S7 e S13 are more potent since determined a decrease in cell number at lower concentrations (10 μM after the first 24 hours of treatment, with a maximum of significant decrease reached with the S13 compound.

Cell cycle and proliferation analysis of S13-treated HT-29 colon cancer cells.

The cytofluorimetry analysis of HT-29 cells showed that 24 hours treatment with 10 μM S13 inhibits the progression of cell cycle. S13-treated cells undergo to a consistent apoptosis after 72 hours of treatment. Moreover,[3H]thymidine uptake assay demonstrated that S13 Src kinase inhibitor significantly inhibited the proliferation of HT-29 cells after 24 and 48 h of culture.

MicroRNA expression induced by S13 in HT-29 colon cancer cells.

Total RNA was extracted from untreated HT-29 cells and from HT-29 cells after 6h and 12h of treatment with 10 μM of S13 and RNA quality control analysis was performed.

High definition Agilent 15K miRNA microarray based on Sanger miRbase12 was hybridised with total RNA and treated cells showed altered expression of several miRNAs.

Among these, has-miR-494 displayed up-regulation in HT-29 cells after 6h of treatment with increased expression at 12h (> 2-fold).

In order to validate the microarray results, the expression levels of 3 most differentially up-regulated miRNAs and 2 most differentially down-regulated miRNAs were examined by qRT-PCR using an All-in-One™ miRNA qRT-PCR Detection Kit (GeneCopoeia, Rockville, MD, USA) and was performed by 7900HT instrument (Applied Biosystems, USA).

Our results showed that the expression levels of hsa-miR-1225, mir-197-3p, and hsa-miR-574-3p were not proven to be significant and not in line with the microarray results, whereas hsa-miR-494, hsa-miR-1207-5p were significantly increased in agreement with microarray data.

These reported preliminary results strongly support the starting hypothesis since 1) the new Src inhibitors inhibited the growth of CD133+ HT-29 CRC cells; 2) they significantly promoted HT-29 apoptosis and inhibited its proliferation; and 3) One of these new Src inhibitors significantly modulated the expression of different miRNAs in HT-29 cells.

Further studies are required to confirm our preliminary data, to clarify the function of miR-494 in HT-29 CRC cells via transfection experiments and its role in transduction pathway downstream of src kinases.

The goal of this study is to identify new drug targets useful for diagnostic and therapeutic purposes in CRC.

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