Novel Src kinase inhibitors inhibit the growth of CD133+ colorectal cancer cells and modulate microRNAs expression

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It is a cancer stem cell concept that cancer cells exhibit a hierarchy, as do normal cells, and that a small fraction of cancer cells are maintained as 'cancer stem cells' that have self-renewal and differentiating abilities. 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. The Src family kinases comprises nine members and among them, Src primarily has been implicated in the development of human cancer. Specifically, in colon cancer, there is a frequent elevation of cellular Src kinase activity over that observed in the adjacent normal mucosa, with activation being linked to malignant potential. Src-family kinases is also involved in stem cell functions and this identifies Src kinases as potential targets for modulating stem cell functions. 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 stem 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+ HT-29 colon cancer 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 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.

Inhibition of proliferation of HT-29 cells by S13 Src kinase inhibitor.

[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 human colorectal cancer cells and from 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 miRbase 12 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).

The reported preliminary results strongly support this 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.