IR Analysis of Leukemic Cells Transduced with an Oncosuppressive Protein Tyrosine Phosphatase by HIV-1 Tat Technology

Giuseppe Bellisola[1,2], Luisa Tomasello[2], Gianfelice Cinque[3], Christophe Sandt[4], Paul Dumas[4], Michela Mirenda[2], Carlo Laudanna[2], and Claudio Sorio[2]

[1]Azienda Ospedaliera Universitaria Integrata Verona, IT
[2]Department of Pathology and Diagnostics, University of Verona, Verona, IT
[3]DIAMOND,beamline MIRIAM, Harwell Science & Innovation Campus, Chilton, UK
[4]SOLEIL, beamline SMIS, L'Orme des Merisiers Saint-Aubin, Gif-sur-Yvette, FR

Protein tyrosine kinases (TKs) and phosphatases (PTPs) control protein tyrosine phosphorylation/dephosphorylation in signalling molecules that regulate the sensitivity of cells to growth factors and to pro-apoptotic stimuli. This homeostasis is subverted in Chronic Myeloid Leukemia (CML) stem cell where the chimeric BCR/ABL fusion gene (Philadelphia chromosome) codifies for Bcr-abl proteins (e.g. p210). Bcr-abl has constitutively increased protein TK activity which blocks cell differentiation and induces cell proliferation. In addition, the expression/activity of receptor-type tyrosine-protein phosphatase gamma (PTPRG) is down regulated, in particular in drug-resistant CML blasts[1]. However, when exposed to tyrosine kinase inhibitors (TKIs), for instance imatinb mesylate (IMA), or transfected with PTPRG, most CML cells can regain the sensitivity to pro-apoptotic stimuli[1].

Aimed at exploring the possibility to restore apoptosis, we transduced the D1-D2 intra-cytoplasmic catalytic domains (ICDs) of human PTPRG fused to HIV-1 Tat penetratin peptide[2] (Tat) into CML cells and we compared the IR spectra of individual cells in untreated controls (CTRLs) and in samples exposed to IMA or to PTP-active rICD-Tat or PTP-inactive rD1028-ICD-Tat proteins, respectively.

Synchrotron Radiation (SR) Fourier transform (FT) InfraRed (IR) microspectroscopy (SR microFTIR) was applied to obtain the spectra of individual cells in samples of human-derived K562-B4 cells with absent PTPRG or of mouse-derived Ba/F3 cells transfected with and stably expressing human p210BCR/ABL fusion gene (wt-Ba/F3). The uptake of rICD-Tat proteins in cells was studied by confocal fluorescence microscopy. The levels of phosphotyrosine were studied in the proteins cell lysates separated by gel electrophoresis and immunoblotted with specific antibodies. PTP activity was measured using para-nitrophenylphosphate (pNPP) substrate or the phosphomolybdate/malachite-green (MG) assay method performed in the presence of ENDpYINASL phosphopeptide. The percentages of viable and apoptotic cells in samples were determined by MTT assay and flow cytometric analysis of fluorescence in cells stained for Annexin-V and Propidium Iodide, respectively.

As compared to untreated CTRLs and CML cells transduced with the rD1028A-ICD-Tat protein, decreased levels of protein phosphotyrosine and increased apoptosis were observed in samples exposed to IMA or to the PTP-active rICD-Tat, respectively. Accurate classification was obtained by hierarchical cluster analysis (HCA) applied to dataset of SR FTIR absorbance spectra.[4]

In conclusion, an active oncosuppressive protein tyrosine phosphatase was efficiently transduced by HIV-1 Tat technology into leukemic cells. PTP-active rICD-Tat protein was able to oppose the abnormal Bcr-Abl TK activity and induce cell apoptosis in CML cells.

References

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