USE OF BLOOD PLATELETS AS siRNA VECTORS FOR SILENCING THE BCR-ABL FUSION GENE IN CHRONIC MYELOID LEUKEMIA (CML)

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Chronic myelogenous (or myeloid or myelocytic) leukemia (CML), is a form of leukemia characterized by the increased and unregulated growth of predominantly myeloid cells in the bone marrow and the accumulation of these cells in the blood. CML is a clonal bone marrow stem cell disorder in which a proliferation of mature granulocytes (neutrophils, eosinophils and basophils) and their precursors is found. It is a type of myeloproliferative disease associated with a characteristic chromosomal translocation called the Philadelphia chromosome.

CML was the first cancer to be linked to a clear genetic abnormality, the chromosomal translocation known as the Philadelphia chromosome. This chromosomal abnormality is so named because it was first discovered and described in 1960 by two scientists from Philadelphia, Pennsylvania, USA: Peter Nowell of the University of Pennsylvania and David Hungerford of Fox Chase Cancer Center.

CML is more common in males than in females (male to female ratio of 1.4:1) and appears more commonly in the elderly with a median age at diagnosis of 65 years. Exposure to ionising radiation appears to be a risk factor, based on a 50 fold higher incidence of CML in Hiroshima and Nagasaki nuclear bombing survivors The rate of CML in these individuals seems to peak about 10 years after the exposure.

CML is often divided into three phases based on clinical characteristics and laboratory findings. In the absence of intervention, CML typically begins in the chronic phase, and over the course of several years progresses to an *accelerated* phase and ultimately to a blast crisis. Blast crisis is the terminal phase of CML and clinically behaves like an acute leukemia.

The BCR-ABL fusion gene is the triggering molecular pathogenic event in chronic myeloid leukemia (CML). The resulting BCR-ABL protein exhibits constitutively active tyrosine kinase activity leading to the permanent activation of several downstream targets resulting in oncogenic growth and inhibition of apoptosis of the leukemic cells.

In recent years, a variety of tyrosine kinase inhibitors (TKIs) targeting BCR–ABL have been developed for the treatment of CML. However, 20–30% of patients develops TKIs resistance and do not respond optimally to TKIs.

Short interfering RNAs (siRNA) have emerged as potential cancer therapeutics and evidence has been provided that siRNAs directed to BCR-ABL reduce protein expression *in vitro* and *in vivo*. A BCR-ABL siRNA was used as a therapeutic approach in an Imatinib-resistant CML patient obtaining inhibition of the BCR-ABL oncogene. However, the patient became resistant after the third siRNA administration suggesting the emergence of siRNA resistance or transfection failure. Indeed, the therapeutic use of naked siRNA is limited due to induction of serum RNase, immune response and by non-targeted effects. Therefore, the development of optimal delivery methods with the use of efficient siRNA carriers is required before getting to the widespread clinical application of siRNA therapeutics *in vivo*. siRNA carriers should cross cell membranes efficiently, have few or no off-target effects, protect siRNAs from degradation/elimination and have low toxicity.

Recently, we have developed a new protocol the trasfection of human platelets with short interfering RNA with high efficiency in few minutes (patent pending).

It's known that platelets can transfer horizontally siRNA molecules towards other cell types, exploiting the interactions between adhesion receptors, so our aim was to employ the original transfection method to introduce siRNAs directed against the BCR-ABL junction sequence in platelets and to use the latter as carriers to transfer the siRNAs to CML cells thus silencing the BCR-ABL fusion transcript.

Blood form a healthy donor was collected and platelets were isolated by washing and subjected to the transfection protocol. The higher transfection rate was achieved after 30 minutes (97%) and maintained for at least 4 hours, using fluorescent siRNA and flow-cytometry analysis.

To evaluate the transfection rate from platelet to other cell types, we perform co-incubation (from 24 to 48 hours) with primary culture cell or cell lines.

Transfected platelet can move horizontally siRNA molecules towards adhesion (HUVEC or HeLa cells) or suspension growing cell (ThP1) with a transfection rate about 70% of positive cells.

Co-incubation experiments were performed by siRNA specific for housekeeping gene HPRT to evaluate down-regulations rate of specific gene target.

We shown that mRNA levels of the gene target HPRT were down-regulated by 50% by RealTime PCR, and also protein levels decreases by 60% by Western Blotting analysis.

Furthermore, we investigated the adhesion receptor involved into platelet-cell interaction related to the siRNA molecules transfer. We shown that cell transfection is inhibited by blocking P-selectin on platelet surface or PSGL-1 on HeLa membrane surface, revealing a key role of this two receptors in the mechanism.

Ph+ K562 cells were selected as *in vitro* model for CML co-incubation experiments and a library of siRNA specific for the fusion site between the two genes ABL and BCR was generated. We evaluated the effect of library members on BCR-ABL mRNA expression level by direct transfection using conventional transfection reagents.

Co-incubation of platelets and K562 cells leads to at most 40% of transfected cells, using a number of platelets 1000-fold higher in relation to K562. Furthermore, the siRNA uptake was not sufficient to induce a down-regulation of BCR-ABL mRNA transcript level.

These unexpected results are due to the K562 membrane wall composition, similar to that of erythrocytes, lacking in adhesion receptor, such as PSGL-1 receptor. This biological morphology of K562 cells does not prepare them to the interaction with platelets.

Currently, we are investigating other Ph+ cell lines with myelocytic membrane morphology, such as EM2, with interesting preliminary result. Our hypothesis is that, in future, this original approach could be based on different circulating blast membrane morphology collected from patients.

Finally, we are studying various murine model to validate our protocol *in vivo*

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