Functional impairment of osteoblasts derived from Shwachman-Diamond syndrome patients: new pharmacological approaches to overcome the genetic mutation.

Shwachman-Diamond syndrome (SDS) is a rare autosomal recessive multi-system disorder characterized by bone marrow failure with predisposition toward myelodysplasia syndrome or acute myeloid leukemia, exocrine pancreatic insufficiency, and skeletal abnormalities. Altered skeletal phenotype is related to abnormal development of the growth plates, delay of secondary ossification centers resulting in metaphyseal dysostosis at femoral head, shortened ribs, costochondral thickening and low turnover osteoporosis with increased risk of fragility fracture.

About 90% of SDS patients carry biallelic inactivating mutations in the SBDS gene, a factor mainly involved in ribosome biogenesis. The most frequent mutations are: the 183-184TA>CT that introduces a premature termination codon (PTC), resulting in the amino-acid change K62X, and the 258+2T>C that affects the donor splice site of intron 2, inducing a frameshift resulting in a PTC (C84fsX3).

The analysis of osteoblasts (OBs) derived from SDS patients compared to oste(57x676)oblasts from healthy, sex and age matched, subjects showed that SDS-OBs display significant differences in the gene expression, particularly in the ossification pathway.

In SDS-OBs the expression of the main genes responsible of osteoblastogenesis, such as Runx2, osterix, osteopontin, bone-sialo protein, osteocalcin, alkaline phosphatase and collagen type I were lower except for osterix, confirmed also at protein level by Western blot analysis.

In addition, SDS-OBs cultured in osteogenic medium displayed impaired mineralization and collagen production, concurring to the early-onset of osteoporosis of SDS patients (ref. 1).

Although some therapeutic strategies are available to overcome the presence of nonsense mutations causing PTCs, no therapeutic option for SDS has been developed so far. A possible strategy involves the incorporation of a random aminoacid at the PTC position through PTC read-through mechanism. PTC read-through can result in the synthesis of a functional full-length protein if the replaced amino is compatible with the protein function. Some aminoglycosides which activate the PTC read-through process, as PTC124 (ataluren), have been approved for the treatment of Duchenne muscular dystrophy. Moreover, ataluren has been recently tested by our collaborators in mesenchymal stem cells derived from SDS patients where it showed an increase in the full-length protein after treatment (ref 2,3).

We therefore hypothesize that the functional impairment of bone cells derived from SDS patients might be improved by the activation of PTC read-through processes. This therapeutic approach might be limited by possible low efficiency of the reconstituted protein. Indeed, the surveillance mechanism of the cells, Nonsense-Mediated mRNA decay (NMD), selectively recognizes and degrades mRNAs whose open reading frame is truncated by the presence of a PTC, in order to protect the cell from accumulating C-terminally truncated proteins with potentially deleterious functions, thus causing a reduced amount of mRNAs to be targeted by aminoglycosides. However, the simultaneous inhibition of NMD by the use of polycyclic compounds, as amlexanox, would increase the PTC read-through process and provide a more efficient therapeutic option.

We plan to clarify the mechanistic role of SDSB in the maintenance of osteoblasts function and we will test whether ataluren can activate a read-through process to promote the production of a full-length and functional SBDS protein, and whether the inhibition of NMD, by amlexanox, might improve the PCT read-through induced by ataluren. Considering that a mouse model recapitulating the most frequent human SDS mutations is not available so far, the usage of human primary cell cultures obtained from SDS patients is essential to test drugs rescuing the genetic mutations.

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References:


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