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Iron-citrate can induce cell senescent phenotype in human fibroblasts *in vitro*

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After a number of replications, human diploid fibroblasts (HDFs) in culture lose the ability to divide, become insensitive to further proliferation and enter a state of replicative senescence (RS) [1]. Subcytotoxic doses of several stressful agents such as hydrogen peroxide, tert-butylhydroperoxide or ethanol, are able to cause stress-induced premature senescence (SIPS) in HDFs *in vitro*. Such senescent cells display many features of RS as growth arrest, senescence associated beta-galactosidase (SA beta-gal), cell enlargement and overexpression of several genes (e.g., p21, TGF beta-1,IGFBP3). During ageing, iron accumulates in several tissues *in vivo*, and also in senescent HDFs *in vitro* [2]. Due to its redox-active properties, it promotes hydroxyl radical production (Fenton reaction) and eventually leads to cell injury. Free radical reactions are known to cause the accumulation of intracellular damage resulting in ageing. Iron may thus be able to cause SIPS. The main objective of the present study was to investigate whether the exposure of HDFs to a subcytotoxic concentration of iron is able to cause SIPS.

WI-38 HDFs at early cumulative population doublings (CPDs ≤ 30) were cultivated in BME with 10% BFS. Treated cells were exposed to 1mM iron-citrate for 24h, whereas control cells were submitted to identical experimental conditions in the absence of the stressful agent. Cell viability and cell proliferation were assessed by MTT assay, SA beta-gal activity was detected as already described [3] and the intracellular iron content was quantified by the BPS assay.
Treated cells presented 96% of cell survival (Fig. 1A) and increased intracellular iron levels when compared with control (Fig 1B), meaning that the dose used was not cytotoxic and that the iron was internalized by the cells. Before stress, fibroblasts presented its typical fusiform shape, however after exposure to iron-citrate cells became larger, resembling the typical senescent morphology (Fig. 2). 72h after stress, cell proliferation was inhibited by 26% when compared with the control (Fig. 3A). Iron stressed cells also presented an increased percentage of cells positive for SA beta-gal (26%) when compared with the lower percentage found for control (Fig. 3B).

As conclusion, these results show that exposure of HDFs to a sublethal dose of iron-citrate induces cell morphology changes which agree with the RS senescent phenotype, suggesting that iron-citrate can induce SIPS. Experiments regarding gene expression are already in progress in order to validate these data.

References

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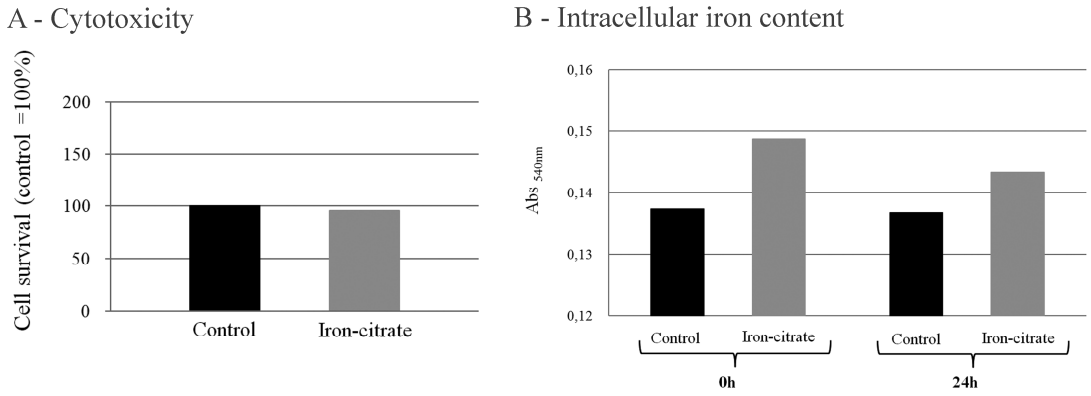

Fig. 1: Cell survival after exposure to 1mM Iron-citrate (A) and intracellular levels of iron 0 and 24h after treatment (B).

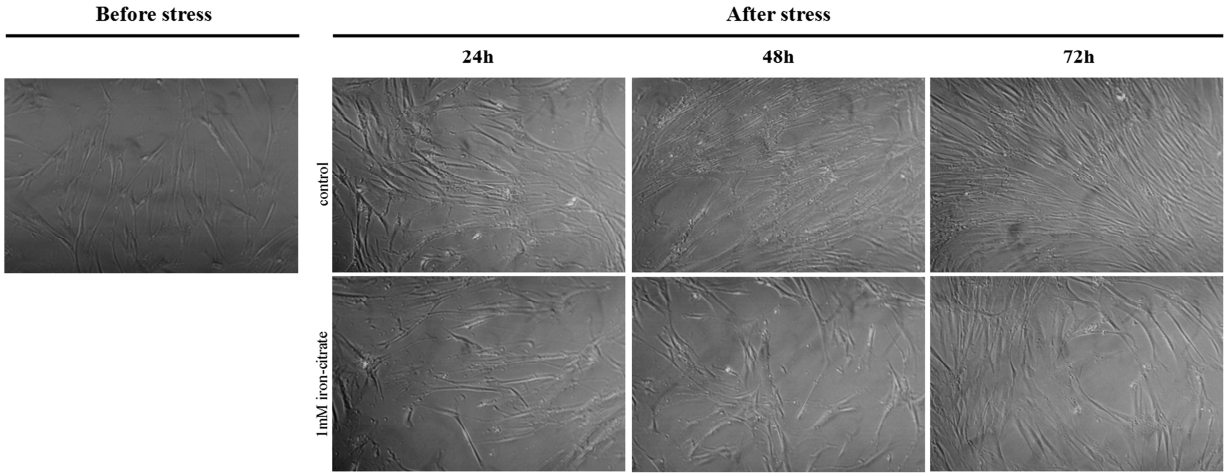

Fig. 2: Morfological changes on cell shape after exposure to 1mM Iron-citrate.

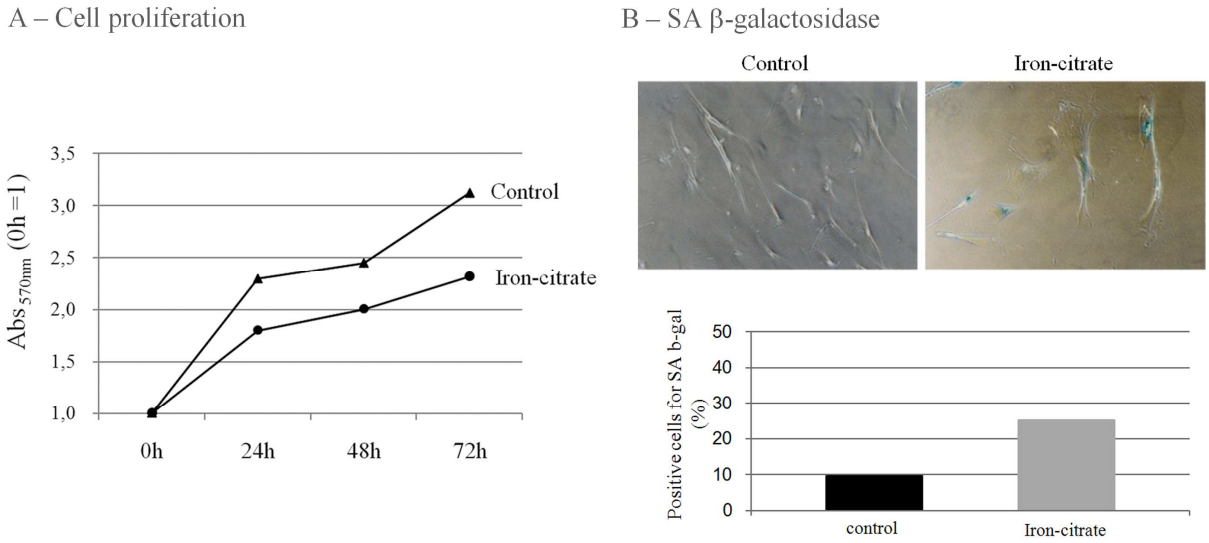

Fig. 3: Cell proliferation was inhibited 72h after exposure to 1mM Iron-citrate (A) and the percentage of SA beta-gal activity positive cells were increased for Iron-citrate cells (B) when compared with control

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