Altered lipid metabolism could drive the bone marrow failure in fanconi anaemia

Fanconi anaemia (FA) is a genetic disease caused by mutations in at least 22 genes. The main clinical aspects are bone marrow (BM) failure and predisposition to develop cancer, particularly acute myeloid leukaemia (AML). Despite numerous studies, the mechanisms responsible for BM failure in FA are not yet well defined. DNA repair has long been considered the main

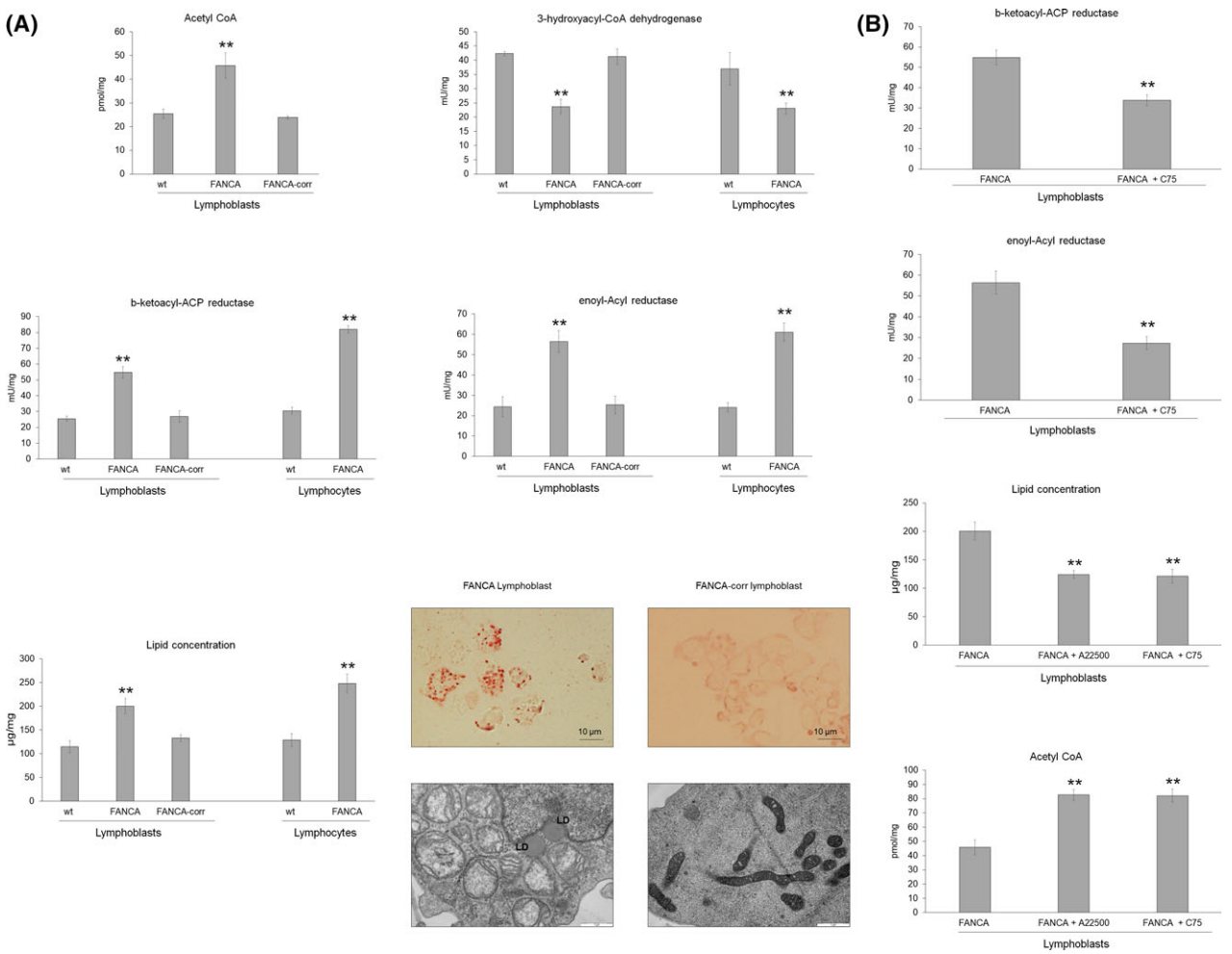

Fig 1. Evaluation of lipid metabolism in FA cells and in lymphocytes from FA patients. (A) Markers of lipid metabolism in wt, Fanconi anaemia (FA) and FA-corr lymphoblasts, as well as in lymphocytes isolated from FA patients and relative control. In particular, the panel reports the Acetyl-CoA concentration and the activities of 3-hydroxyacyl-CoA dehydrogenase, as markers of beta-oxidation, and β-ketoacyl-ACP reductase and Enoyl-ACP-reductase assay, as markers of fatty acid synthesis, and the lipid content. Data are representative of five independent experiments and are expressed as mean ± SD. **indicates a significant difference for $P < 0{\cdot}01$ between FA lymphoblasts or FA lymphocytes with respect to the control samples. The presence of lipid droplets in FA lymphoblasts has been estimated by oil red staining and electron microscopy. (B) The effect of treatment with C75 (4-methylene-2-octyl-5-oxotetra- hydrofuran-3-carboxylic acid), inhibitors of fatty acid synthesis on the activities of β-ketoacyl-ACP reductase and Enoyl-ACP-reductase in FA lymphoblasts. Moreover, the effects A922500, a specific inhibitor of triglycerides synthesis, were also evaluated on the lipid content and the Acetyl-CoA concentration. Data are representative of five independent experiments and are expressed as mean ± SD. **significant difference ($P < 0{\cdot}01$) between treated and untreated FA lymphoblasts. The enzymatic activities are expressed as mUnits/mg (mU/mg), which correspond to the nmoles of substrate catalysed in 1 min. FANCA, Fanconi anaemia complementation group A; FANCA-corr, Fanconi anaemia complementation group A-corrected; LD, lipid droplets; wt, wild type. [Colour figure can be viewed at wileyonlinelibrary.com]

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molecular hallmark of FA cells. However, in recent years, other aberrant cellular pathways have emerged as important contributors to the pathogenesis of the disease. Among these, mitochondria dysfunction and oxidative stress were shown to have a role in the deranged functioning of FA cells (Du *et al*, 2008). In particular, it appears that a defect of the respiratory chain in FA cells increases oxidative stress and that this is associated with glucose dysmetabolism and insulin resistance, chromosome instability, reduced metalloproteases activity and cytotoxic effects of tumour necrosis factor-alpha. Recently it has been shown that FA cells try to compensate for the mitochondria impairment by enhancing the glycolysis pathway, although this metabolic shift does not completely restore the energetic status (Cappelli *et al*, 2017).

Lipid metabolism, which is mainly composed of beta-oxidation and lipid synthesis according to the energetic need of the cell, is another pathway involved in cellular energy balance. Normally, high-energy demand enhances beta-oxidation, whereas high ATP levels stimulate lipogenesis. However, in the case of mitochondria dysfunction and oxidative stress, an increment of lipid metabolism and lipid droplet accumulation occur which, in turn, generate an increase in oxidative stress production (Furukawa *et al*, 2004), in a self-sustained negative loop. Of note, about 50% of FA patients develop lipid metabolism disorder (Giri *et al*, 2007). Lipid metabolism of FA cells has not been investigated to date. In this study we evaluated how the synthesis and beta-oxidation of fatty acids are modulated in FA cells. The Materials and Methods are detailed in Appendix S1.

We have previously demonstrated that FA cells preferentially convert glucose to lactate rather than use it for the aerobic metabolism, despite the normal activity of pyruvate dehydrogenase (Cappelli *et al*, 2017). However, when analysing the intracellular concentration of Acetyl CoA (AcCoA), we have observed that FA cells displayed a higher concentration with respect to the control and corrected samples (Fig 1A). Given that AcCoA is produced not only by aerobic glucose catabolism, but is also formed by beta-oxidation of fatty acid, we evaluated the activity of 3-hydroxyacyl-CoA dehydrogenase, and observed that FA cells displayed a lower activity compared to controls and corrected samples (Fig 1A). This suggests that the increased AcCoA content in FA cells is not due to the increased fatty acid oxidation, but may be dependent on impairment of the oxidative phosphorylation activity. In other words, the defect in the electron transfer between complexes I and III may determine a slowing of the Krebs cycle flux, causing AcCoA accumulation.

However, considering that cells need to recycle CoA, to maintain a constant availability, we have evaluated the activities of β-ketoacyl-ACP reductase and enoyl-Acyl reductase, two enzymes involved in fatty acids synthesis. Data show that this metabolism appeared to be higher in FA cells compared to control and corrected samples, determining a fatty acid lipid accumulation (Fig 1A). Normally, excess lipids are stored in the cell as triglycerides, and then packaged into cytoplasmic lipid droplets (LDs) to protect the cell against lipotoxicity (Listenberger *et al*, 2003). Red oil staining and electron

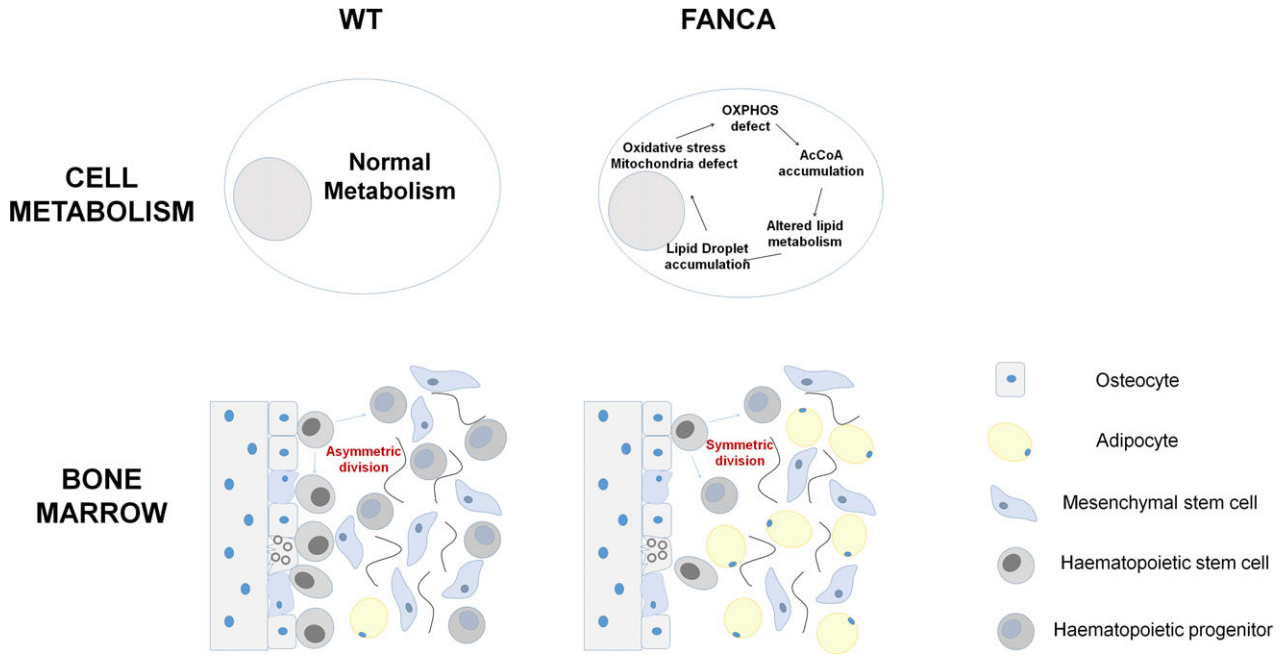

Fig 2. Correlation between lipid metabolism alteration and haematopoietic stem cells differentiation. The scheme shows the correlation between altered lipid metabolism and haematopoietic stem cells (HSC) decline in Fanconi anaemia (FA) cells with respect to a healthy sample. In particular, the literature reports that an increased population of adipocytes has been observed in the bone marrow of FA patients probably as a consequence of FA mesenchymal stem cells preferentially differentiating as adipocytes, which are deleterious for HSC self-renewal (Zhou *et al*, 2017). AcCoA, Acetyl-CoA; FANCA, Fanconi anaemia complementation group A; OXPHOS, oxidative phosphorylation; WT, wild type. [Colour figure can be viewed at wileyonlinelibrary.com]

microscopy of FA lymphoblasts showed an increment in LDs compared to FA corrected cells, confirming an excess of lipids production in FA cells (Fig 1A).The results on enzymatic activity and cell lipid content were confirmed in primary lymphocytes obtained from FA patients (Fig 1A) and in Fanc-D2 cell lines (data not shown).

We obtained further confirmation of our results using C75 (4-methylene-2-octyl-5-oxotetra- hydrofuran-3-carboxylic acid), an inhibitor of fatty acid synthesis, and A922500, an inhibitor of triglycerides synthesis. In particular, FA cells treated with C75 show a reduction of β-ketoacyl-ACP reductase and enoyl-Acyl reductase (Fig 1B). Moreover, both C75 and A922500 produced about a 30% decrease in lipid content and an increase in AcCoA content (Fig 1B), confirming that FA cells convert the excess of AcCoA to fatty acids, which are accumulated in LDs.

From our results it appears evident that lipid metabolism is altered in FA cells. Interestingly this finding suggests that the decline in haematopoietic stem cells (HSC) could be correlated with the alteration of this metabolic pathway (Fig 2). In this regard, an increased population of adipocytes has been observed in the BM of FA patients and mouse models (Pulliam-Leath *et al*, 2010), probably as a consequence of FA mesenchymal stem cells preferentially differentiating as adipocytes (Zhou *et al*, 2017), which appear deleterious for HSC self-renewal (Naveiras *et al*, 2009). Moreover, *PPARG*, a key regulator of adipocyte differentiation and lipid synthesis, is up-regulated in $CD34^+$ cells from FA patients (Sertorio *et al*, 2017), confirming the deregulation in FA cells of lipid metabolism.

Finally, our observation of reduced fatty acid beta-oxidation in FA cells represents an important event in HSC renewal, considering that the reduction of beta oxidation has an important role in self-renewal asymmetric division of HSC (Ito *et al*, 2012) and in driving HSC differentiation.

Based on these data, we conclude that the interest on biochemical metabolism does not represent only a scientific curiosity in FA cells, but that it is strongly connected with the clinical aspects of this disease and may suggest relevant clues in diseases prone to BM failure.

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Author contributions

S.R. and P.D. designed the research, F.S., M.C. and SR. performed the experiments, C.D. and E.C. wrote the manuscript.

Disclosure statement

No Authors have conflict of interest.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Materials and methods.

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