Abstract

The resistance to tamoxifen, a drug used in the adjuvant therapy for hormone sensitive breast cancer, represents a major clinical obstacle. Although various mechanisms leading to tamoxifen resistance have been described and intensively studied, a significant number of patients still become resistant to the treatment and eventually relapse.

Tamoxifen therapy has been shown to enrich tumors with cancer stem cells (CSCs), which are naturally resistant, and have self-renewal ability and the potential to form secondary tumors. Metabolic rewiring, altered iron metabolism and upregulation of ATP-binding cassette (ABC) transporters have been shown to be important in the maintenance of CSC phenotype. Therefore, we investigated these mechanisms as possible contributors to tamoxifen resistance *in vitro* in two tamoxifen resistant (Tam5R) cell lines that we established.

We show that Tam5R cells have dramatically disassembled and less active mitochondrial supercomplexes (SCs) and higher level of mitochondrial superoxide, together with a fragmented mitochondrial network. Such dysfunction of mitochondria results in the AMP-activated protein kinase (AMPK) activation and metabolic rewiring towards glycolysis. Importantly, cells lacking functional mitochondria are significantly more resistant to tamoxifen, supporting a role of mitochondria in tamoxifen resistance.

Further, our analysis revealed significant changes in proteins participating in iron uptake, storage, export and iron sensing as well as in iron-sulfur (Fe-S) cluster assembly and hypoxia response in Tam5R cells. In addition, less incorporation of ^{55}Fe into Fe-containing mitochondrial proteins was detected. Therefore, we propose that altered iron trafficking and utilization in Tam5R cells may be linked with the resistant phenotype.

Finally, the expression profile of ABC transporters, well described contributors to multidrug resistance, was altered in Tam5R cells, with similar change in protein level of ABCC5, ABCG1 and ABCF2 in both cell lines, thereby suggesting their possible role in tamoxifen resistance.

Key words: breast cancer, tamoxifen resistance, mitochondria, iron metabolism, ABC transporters