LETTER

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A metabolic function of *FGFR3–TACC3* gene fusions in cancer

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Chromosomal translocations that generate in-frame oncogenic gene fusions are notable examples of the success of targeted cancer therapies[1–3]. We have previously described gene fusions of *FGFR3-TACC3* (F3–T3) in 3% of human glioblastoma cases[4]. Subsequent studies have reported similar frequencies of F3–T3 in many other cancers, indicating that F3–T3 is a commonly occuring fusion across all tumour types[5,6]. F3–T3 fusions are potent oncogenes that confer sensitivity to FGFR inhibitors, but the downstream oncogenic signalling pathways remain unknown[2,4–6]. Here we show that human tumours with F3–T3 fusions cluster within transcriptional subgroups that are characterized by the activation of mitochondrial functions. F3–T3 activates oxidative phosphorylation and mitochondrial biogenesis and induces sensitivity to inhibitors of oxidative metabolism. Phosphorylation of the phosphopeptide PIN4 is an intermediate step in the signalling pathway of the activation of mitochondrial metabolism. The F3–T3–PIN4 axis triggers the biogenesis of peroxisomes and the synthesis of new proteins. The anabolic response converges on the PGC1α coactivator through the production of intracellular reactive oxygen species, which enables mitochondrial respiration and tumour growth. These data illustrate the oncogenic circuit engaged by F3–T3 and show that F3–T3-positive tumours rely on mitochondrial respiration, highlighting this pathway as a therapeutic opportunity for the treatment of tumours with F3–T3 fusions. We also provide insights into the genetic alterations that initiate the chain of metabolic responses that drive mitochondrial metabolism in cancer.

To investigate the transcriptional changes elicited by F3–T3, we expressed F3–T3 in immortalized human astrocytes and compared gene expression profiles of cells treated with a specific inhibitor against FGFR tyrosine kinase (TK, PD173074) or vehicle. Human astrocytes expressing F3–T3 were also compared to human astrocytes that expressed kinase-dead F3–T3 (F3–T3(K508M)) or were transduced with an empty vector (Extended Data Fig. 1a). Hierarchical clustering based on genes that were differentially expressed between F3–T3 human astrocytes and PD173074-treated F3–T3 cells showed that F3–T3 human astrocytes differed from the other three groups (Fig. 1a and Extended Data Fig. 1b). Analysis of a Gene Ontology enrichment map showed that, in addition to the expected enrichment for mitotic activity[4], oxidative phosphorylation and mitochondrial biogenesis were the most significant categories to be enriched in F3–T3 human astrocytes for each of the three independent comparisons (Fig. 1b, Extended Data Fig. 1c and Supplementary Table 1). We confirmed the expression changes of mitochondrial genes by quantitative PCR with reverse transcription (RT–qPCR) (Extended Data Fig. 1d).

Compared to human astrocytes expressing the empty vector or F3–T3(K508M), F3–T3 human astrocytes exhibited increased levels of mitochondrial DNA, mitochondrial mass (MitoTracker Red) and produced higher levels of ATP (Fig. 1c, d and Extended Data Fig. 1e). F3–T3 increased respiratory complex proteins (SDHB, UQCRC1 and ATP5A1) and the mitochondrial membrane transporter VDAC1 (Extended Data Fig. 1f). We also found higher levels of VDAC1 and NDUFS4 in tumours generated from mouse glioma stem cells (mGSCs) expressing human F3–T3 and small hairpin RNA (shRNA) against *Trp53* (shTrp53) (hereafter F3–T3;shTrp53) than in tumours formed by mGSCs expressing oncogenic HRAS(12V) and shTrp53 (hereafter HRAS(12V);shTrp53)[4,7] (Extended Data Fig. 1g). Introduction of F3–T3 in human astrocytes, RPE and U251 cells increased the basal and maximal oxygen consumption rate (OCR) of these cells compared to cells transduced with F3–T3(K508M) or empty vector and this effect was reversed by FGFR-TK inhibition with AZD4547 in cells expressing exogenous F3–T3 and human glioblastoma (GBM)-derived GSC1123 cells with endogenous F3–T3[4] (Fig. 1e and Extended Data Fig. 2a–d). F3–T3 elicited only a mild increase in the extracellular acidification rate (ECAR), leading to an increase in the OCR:ECAR ratio (Extended Data Fig. 2e, f). After treatment with the inhibitor of ATP synthase oligomycin, F3–T3 human astrocytes displayed reduced ATP levels and cell growth (by more than 70%) but were resistant to the substitution of glucose with galactose in the culture medium, a condition that imposes oxidative metabolism and markedly affected cell growth of human astrocytes treated with vector (Extended Data Fig. 2g, h). A 72-h treatment with the mitochondrial inhibitors metformin, menadione or tigecycline impaired growth of GSC1123 cells but was ineffective in GSC308 F3–T3-negative gliomaspheres[4] (Fig. 1f). Similarly, mitochondrial inhibitors reduced the viability of F3–T3;shTrp53 mGSCs but did not affect HRAS(12V);shTrp53 mGSCs (Fig. 1g and Extended Data Fig. 2i–k). However, tigecycline decreased COX1 and COX2, two respiratory complex subunits translated by mitochondrial ribosomes[8], and mitochondrial inhibitors reduced ATP production, indicating that these compounds were similarly active in both cell types (Extended Data Fig. 2l, m). We also found that treatment with tigecycline (50 mg kg^{-1}) suppressed tumour growth of F3–T3;shTrp53 mGSCs glioma xenografts with a more than 50% reduction in tumour

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