Stem cells: Mitochondrial biogenesis links growth and EGFR signaling

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Stem cells: Mitochondrial Biogenesis is a Missing Link Between Growth and EGFR Signaling

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The EGFR pathway is central to cell proliferation, growth, and survival and is often deregulated in cancers. A new study links downstream effectors of EGFR to stem cell growth via mitochondrial biogenesis and metabolic reprogramming.

Long-term tissue regeneration relies on the capacity of adult stem cells to adapt their self-renewal and differentiation in response to damage and microenvironment alteration. EGFR signaling controls many biological processes essential to stem cells' ability to maintain tissue integrity including proliferation, growth, differentiation, migration, and inhibition of apoptosis. Abnormal activation of EGFR, or of downstream constituents of the pathway such as Ras or Raf, are oncogenic drivers of several types of cancers including breast cancer, non-small-cell lung cancer, metastatic colorectal cancer, glioblastoma and pancreatic cancer. Despite this documented role of EGFR pathway in normal stem cell activity and in contexts of tumorigenesis, the mechanisms of its action remain unclear. Notably, the downstream targets of activated EGFR are only partially defined and completing this knowledge could provide novel therapeutic targets. A new study in this issue of Current Biology from **Zhang *et al*., 2022**[1] helps to fill this gap in our understanding through identifying additional downstream effectors of the EGFR/Ras/ERK pathway in the context of stem cell growth and proliferation using *Drosophila* adult intestinal stem cells as a model system.

Previous studies on the EGFR pathway in the adult *Drosophila* intestinal epithelium demonstrated that it is necessary and sufficient to induce intestinal stem cell (ISC) proliferation after intestinal damage, enteric infection, or oxidative stress[2,3]. This role of EGFR in tissue regeneration is very similar to its role in the mammalian intestine after damage[4]. Zhang *et al*., took advantage of the well-described hyperplastic phenotype induced in the midgut by ectopic activation of EGFR[3] to characterize the EGFR-mediated stem cell response and identify downstream mechanisms contributing to such response. They report that increased stem cell growth accompanies the previously described ISC proliferation upon activation of EGFR signalling in multiple contexts including enteric bacterial infection, expression of the EGFR ligand, Spitz, or the downstream transcription factors Pnt and Ets21C. Interestingly, stem cell

growth appears to be independent of cell division since EGFR activation and a concomitant blockage of the cell cycle in ISCs did not prevent growth.

In order to identify mechanisms downstream of EGFR controlling both ISC growth and proliferation, Zhang and colleagues combined transcriptomic, genomic and metabolomic approaches. RNA-Seq on ISCs overexpressing Pnt, Ets21C, or Spitz, revealed that, in addition to the deregulation of genes linked to cell cycle, DNA replication or cell death, numerous genes related to mitochondrial metabolic processes are up-regulated. Their data suggest direct transcriptional regulation of mitochondrial metabolism downstream of EGFR as many genes linked to oxidative phosphorylation (OXPHOS) and tricarboxylic acid (TCA) cycle are bound by Pnt and Ets21C in ISCs as determined by DamID-Seq profiling. Two additional recent studies on *Drosophila* ISCs[5] and regenerating imaginal discs[6] also identified Ets21C targets, though downstream of the JNK pathway instead of EGFR. Future studies will undoubtedly shed light on whether deregulated genes in these contexts are shared or pathway-specific, as well as establish whether JNK could also regulate mitochondrial processes.

The study of Zhang *et al*. further demonstrates that EGFR activation is able to increase mitochondrial activity not only through the direct activation of the expression of genes controlling mitochondrial metabolism, but also indirectly, through expression of the conserved mitochondrial biogenesis factor mtTFB2. Interestingly, they propose that mtTFB2 is directly activated downstream of EGFR by the transcription factor Pnt, a *Drosophila* homolog of the human Nuclear Respiratory Factor 2 (NRF-2), a known regulator of mtTFB2 expression and mitochondrial biogenesis in mammals[7]. Thus, the findings of Zhang *et al.* highlight a direct link between transcriptional output of EGFR signaling and regulation of a major mitochondrial biogenesis factor. Is control of mitochondrial biogenesis by EGFR conserved? Using human cell culture experiments, the authors provide evidence that the EGFR pathway can also increase mitochondrial mass, although independently of MEK in this context. This raises the question whether additional pathways known to regulate mitochondrial biogenesis and known to be activated downstream of EGFR, such as AMP-Activated Protein Kinase (AMPK) or mechanistic Target Of Rapamycin (mTOR)[8], could be involved. In addition, whether NRF-2 acts downstream of EGFR in mammals, is not known. The work of Zhang *et al.*, suggesting a possible conservation in mammals, calls for further investigation on the effects of EGFR and its downstream effectors on mitochondrial biogenesis and activity in other systems.

What is the metabolic impact of EGFR-driven mitochondrial biogenesis? Lipid and metabolite profiling on the intestine after overexpression of the EGFR ligand Spitz in stem cells confirmed a reconfiguration of cell metabolism with an increase in glycolysis and mitochondrial

processes such as OXPHOS, TCA cycle and fatty acid β-oxidation. The authors propose that glycolysis would be favored by the cell to produce nucleotides and amino acids while fatty acid consumption through ß-oxydation would be used to provide acetyl-CoA to the TCA cycle in order to generate energy (ATP, NADH; Figure 1). This EGFR-dependent metabolic switch is concomitant with the transition of stem cells from a relatively quiescent state to a highly proliferative one, requiring a burst of nucleotides, amino acids and energy. Furthermore, this is reminiscent of the metabolic switch observed in mammalian hematopoietic stem cells that are quiescent in the bone marrow, but highly proliferative upon exiting the niche and in which mitochondrial metabolism and respiration are simultaneously activated[9,10]. Over the past decade, several other studies highlighted the role of mitochondrial metabolism changes in stem cell function and fate[11,12]. However, the implication of EGFR pathway in this process is, to our knowledge, undocumented. Further studies should elucidate whether a switch between metabolic configurations is regulated by EGFR in other contexts and in other stem cell types.

Mitochondrial metabolism is a well-known participant in metabolic reprogramming involved in tumor initiation, growth, and metastasis[13]. Abnormal activation of EGFR/Ras/MAPK pathway is also a common feature of many types of cancer. Similar to the observations of Zhang and colleagues, enzymes involved in glycolysis, mitochondrial OXPHOS and the TCA cycle have been found to be essential for tumor formation in cancer models driven by oncogenic *KRAS* and loss of *p53*[14,15]. Together, these data raise the question of a similar role of EGFR activation on metabolic reprogramming in oncogenic conditions.

While Zhang *et al.* have identified mitochondrial metabolism as important downstream of EGFR signaling, its precise roles on ISC proliferation remain to be further clarified. For example, a metabolic state called the "Warburg effect" in which aerobic glycolysis is favored over mitochondrial activity is characteristic of many tumors. The Warburg effect has been also reported in aging stem cells in humans[16], aging *Drosophila* ISCs[17], as well as ISCs overexpressing oncogenic Ras^{v12} [17] where it correlates with enhanced stem cell proliferation. While both studies show an increase of ISC proliferation[16,17], the metabolic switch during aging in ISCs appears distinct from the one described by Zhang *et al.*, which consists of a concomitant increase of glycolysis, ß-oxidation in addition to other mitochondrial metabolism pathways upon EGFR activation. Zhang *et al*., further propose that this variant of the Warburg effect induced by EGFR signaling, involves glucose consumption through aerobic glycolysis to provide nutrients essential to biomass increase while cell demands for energy would rely on fatty acid ß-oxidation and the TCA cycle.

Interestingly, other studies further reported that altering mitochondria homeostasis either promoting mitochondrial biogenesis or preventing mitochondrial degradation by mitophagy in *Drosophila* ISCs prolongs lifespan and prevents intestinal dysplasia during aging[18,19]. In particular, overexpression of the master regulator of mitochondrial biogenesis (*PGC-1*)[18] or increasing mitochondrial electron transport chain activity[20], prevent ISC over-proliferation during aging. Thus, the effects on stem cell proliferation in these contexts are different than in that of EGFR activation. Such differences in metabolic and stem cell responses to increased mitochondrial activity remain unresolved although could be due to other downstream effectors of EGFR, notably the direct effect on cell cycle gene expression independent of mitochondrial metabolism.

Further efforts to gain understanding into this new link between EGFR pathway and mitochondrial regulation could yield a deeper understanding of how stem cells activate a metabolic switch to maintain tissue integrity and as well as how this mechanism may be highjacked in tumorigenic conditions.

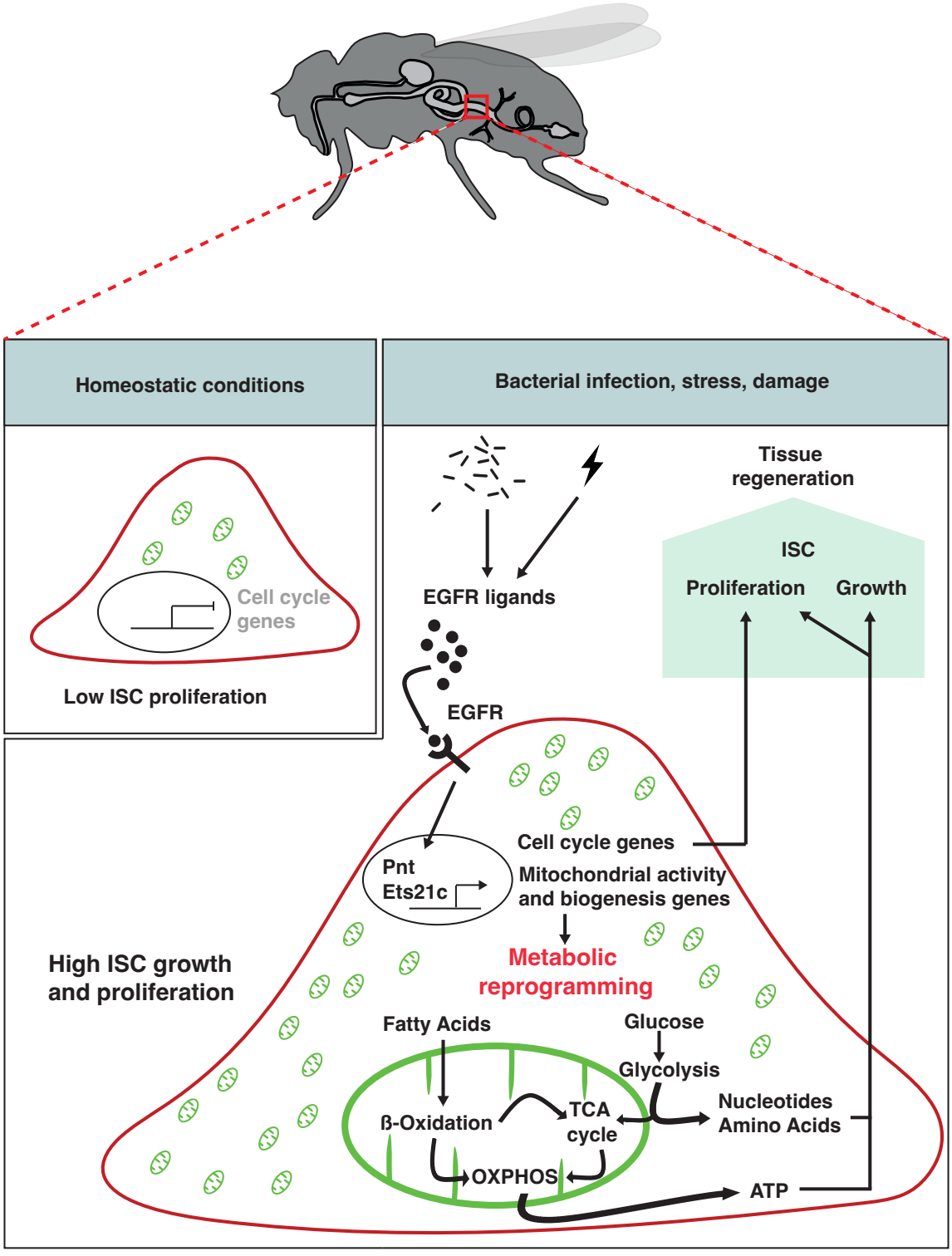
Figure legend.

Figure 1. EGFR-driven mitochondrial metabolic switch in *Drosophila* intestinal stem cells

Under normal physiological conditions, intestinal stem cells (ISCs) divide slowly, replacing differentiated cells to maintain intestinal function and structure throughout the life of the organism. Upon bacterial infection, stress, or damage to the tissue, EGFR ligands are secreted by the enterocytes or the progenitor cells (ISCs and enteroblasts) as well as the visceral muscle surrounding the gut. EGFR/Ras/MAPK pathway activity in ISCs leads to expression of the transcription factors Pnt and Ets21c, which in turn, control genes regulating the cell cycle, mitochondrial activity and biogenesis genes. This increase in mitochondrial activity leads to a metabolic reprogramming essential both for ISC growth and proliferation required for proper tissue regeneration.

1.
2. Buchon, N., Broderick, N.A., Kuraishi, T., and Lemaitre, B. (2010). Drosophila EGFR pathway coordinates stem cell proliferation and gut remodeling following infection. BMC Biol. *8*, 152.
3. Jiang, H., Grenley, M.O., Bravo, M.-J., Blumhagen, R.Z., and Edgar, B.A. (2011). EGFR/Ras/MAPK Signaling Mediates Adult Midgut Epithelial Homeostasis and Regeneration in Drosophila. Cell Stem Cell *8*, 84–95.
4. Jardé, T., Chan, W.H., Rossello, F.J., Kaur Kahlon, T., Theocharous, M., Kurian Arackal, T., Flores, T., Giraud, M., Richards, E., Chan, E., et al. (2020). Mesenchymal Niche-Derived Neuregulin-1 Drives Intestinal Stem Cell Proliferation and Regeneration of Damaged Epithelium. Cell Stem Cell *27*, 646-662.e7.
5. Mundorf, J., Donohoe, C.D., McClure, C.D., Southall, T.D., and Uhlirova, M. (2019). Ets21c Governs Tissue Renewal, Stress Tolerance, and Aging in the Drosophila Intestine. Cell Rep. *27*, 3019-3033.e5.
6. Worley, M.I., Everetts, N.J., Yasutomi, R., Chang, R.J., Saretha, S., Yosef, N., and Hariharan, I.K. (2022). Ets21C sustains a pro-regenerative transcriptional program in blastema cells of Drosophila imaginal discs. Curr. Biol., S0960982222010028.
7. Yang, Z.-F., Drumea, K., Mott, S., Wang, J., and Rosmarin, A.G. (2014). GABP Transcription Factor (Nuclear Respiratory Factor 2) Is Required for Mitochondrial Biogenesis. Mol. Cell. Biol. *34*, 3194–3201.
8. Ploumi, C., Daskalaki, I., and Tavernarakis, N. (2017). Mitochondrial biogenesis and clearance: a balancing act. FEBS J. *284*, 183–195.
9. Takubo, K., Nagamatsu, G., Kobayashi, C.I., Nakamura-Ishizu, A., Kobayashi, H., Ikeda, E., Goda, N., Rahimi, Y., Johnson, R.S., Soga, T., et al. (2013). Regulation of Glycolysis by Pdk Functions as a Metabolic Checkpoint for Cell Cycle Quiescence in Hematopoietic Stem Cells. Cell Stem Cell *12*, 49–61.
10. Yu, W.-M., Liu, X., Shen, J., Jovanovic, O., Pohl, E.E., Gerson, S.L., Finkel, T., Broxmeyer, H.E., and Qu, C.-K. (2013). Metabolic Regulation by the Mitochondrial Phosphatase PTPMT1 Is Required for Hematopoietic Stem Cell Differentiation. Cell Stem Cell *12*, 62–74.
11. Cliff, T.S., and Dalton, S. (2017). Metabolic switching and cell fate decisions: implications for pluripotency, reprogramming and development. Curr. Opin. Genet. Dev. *46*, 44–49.
12. Tsogtbaatar, E., Landin, C., Minter-Dykhouse, K., and Folmes, C.D.L. (2020). Energy Metabolism Regulates Stem Cell Pluripotency. Front. Cell Dev. Biol. *8*, 87.
13. Martínez-Reyes, I., and Chandel, N.S. (2021). Cancer metabolism: looking forward. Nat. Rev. Cancer *21*, 669–680.
14. Zhu, X.G., Chudnovskiy, A., Baudrier, L., Prizer, B., Liu, Y., Ostendorf, B.N., Yamaguchi, N., Arab, A., Tavora, B., Timson, R., et al. (2021). Functional Genomics In Vivo Reveal Metabolic Dependencies of Pancreatic Cancer Cells. Cell Metab. *33*, 211-221.e6.
15. Biancur, D.E., Kapner, K.S., Yamamoto, K., Banh, R.S., Neggers, J.E., Sohn, A.S.W., Wu, W., Manguso, R.T., Brown, A., Root, D.E., et al. (2021). Functional Genomics Identifies Metabolic Vulnerabilities in Pancreatic Cancer. Cell Metab. *33*, 199-210.e8.
16. Short, K.R., Bigelow, M.L., Kahl, J., Singh, R., Coenen-Schimke, J., Raghavakaimal, S., and Nair, K.S. (2005). Decline in skeletal muscle mitochondrial function with aging in humans. Proc. Natl. Acad. Sci. *102*, 5618–5623.
17. Morris, O., Deng, H., Tam, C., and Jasper, H. (2020). Warburg-like Metabolic Reprogramming in Aging Intestinal Stem Cells Contributes to Tissue Hyperplasia. Cell Rep. *33*, 108423.
18. Rera, M., Bahadorani, S., Cho, J., Koehler, C.L., Ulgherait, M., Hur, J.H., Ansari,

W.S., Lo Jr, T., Jones, D.L., and Walker, D.W. (2011). Modulation of Longevity and Tissue Homeostasis by the Drosophila PGC-1 Homolog. Cell Metab. *14*, 623–634.
19. Koehler, C.L., Perkins, G.A., Ellisman, M.H., and Jones, D.L. (2017). Pink1 and Parkin regulate Drosophila intestinal stem cell proliferation during stress and aging. J. Cell Biol. *216*, 2315–2327.
20. Hur, J.H., Bahadorani, S., Graniel, J., Koehler, C.L., Ulgherait, M., Rera, M., Jones, D.L., and Walker, D.W. (2013). Increased longevity mediated by yeast NDI1 expression in Drosophila intestinal stem and progenitor cells. Aging *5*, 662–681.


Homeostatic conditions
Bacterial infection, stress, damage
Cell cycle genes
Low ISC proliferation
EGFR ligands
Tissue regeneration
ISC
Proliferation
Growth
EGFR
Pnt
Ets21c
Cell cycle genes
Mitochondrial activity and biogenesis genes
Metabolic reprogramming
High ISC growth and proliferation
Fatty Acids
Glucose
Glycolysis
ß-Oxidation
TCA cycle
Nucleotides
Amino Acids
OXPHOS
ATP