低氧壓力與胰島素生長因子第一型訊息之交互作用對小鼠精原幹細胞的多功能性之探討

Cross-talking of Hypoxia Stress and IGF-1 Signaling in Pluripotent Regulation of Mouse Germline Stem Cells

中文摘要

癌幹細胞 (cancer stem cells, CSCs) 被認爲存在於腫瘤細胞之中，而關於 CSCs 的起源，則不清楚。目前 CSCs 被認爲可能是來自於正常的幹細胞 (例如 glioma CSCs 和 intestine CSCs)，因爲基因的不穩定性或是導因於微環境 (niche) 中對細胞的壓力 (如低氧，hypoxia) 所產生的刺激，可能是誘使幹細胞轉形 (transformation) 變成 CSCs 的原因。然而，目前關於低氧環境下誘導幹細胞轉形爲癌幹細胞之相關機轉的研究，仍非常有限。臨床疾病中，我們可以發現在 human pluripotent testicular tumors (包括 seminomas 和 embryonal carcinomas) 的組織中，表現大量 HIF-1α、HIF-2α和 IGF-1 蛋白，高度暗示著在多功能幹細胞睪丸癌中，hypoxia stress 與 IGF-1 signaling 間的交互作用。我們實驗室已成功的建立了 serum-free stem-niche cell 共同培養的系統，能將新生小鼠的睪丸細胞在體外培養成多功能性精原幹細胞 (AP+GSCs)；並且藉此發現 IGF-1/IGF-1R signaling 可以維持 AP+GSCs 的多功能性。有趣的是，當與 normoxia (21 % O_2) 相對照，可以發現在 hypoxia (5 % O_2) 培養下的 AP+GSCs，表現高度鹼性磷酸酶活性 (AP)、細胞增生速率、HIF-1α、HIF-2α 和 Oct-4 蛋白以及 pluripotent gene (像是 Oct-4、Sox2、Nanog、Klf4 和 c-Myc) 的表現。除此之外，IGF-1 和 IGF-1R 在 hypoxia 下的表現量也都會增加。進一步給與 PPP (IGF-1R 的抑制劑) 和 LY294002 (PI3K 的抑制劑) 於 AP+GSCs 培養液中，發現 Oct-4、HIF-1α和 HIF-2α的蛋白表現量都會有顯著的下降，暗示 IGF-1 signaling 可以調控 Oct-4 和 HIF-2α的表現。綜合以上結果，證明了 hypoxia 會與 IGF-1 signaling 共同調控 AP+GSCs 之 stemness (Oct-4、Sox2、Nanog 和 Klf4) 與 tumor factor (c-Myc) 的表現，促使幹細胞在 hypoxia 下進行轉化 (transformation)。

英文摘要

Recent advances in cancer research suggest that existance of cancer stem cells (CSCs) in tumor tissues. The origin of CSCs are still controversial; but it has been proved CSCs are possibly originated from organ stem cells (e.q. glioma CSCs and intestine CSCs). The niche stress such as hypoxia effect may provide external signals in stem cell transformation. However, the mechanism of hypoxia-induced stem cell transformation still remains unclear. Our preliminary observations in human pluripotent testicular tumors (seminomas and embryonal carcinomas) showed a high

level of hypoxia-inducible protein HIF-1α/HIF-2α as well as IGF protein expression in tissues. This observation strongly highlights the cross-talking of niche hypoxic stress and IGF-1 signaling in transformation of germline stem cells into pluripotent cancers. To address this point, we have successfully established a serum-free stem-niche cell co-culture system to generate pluripotent germline stem cells from neonatal mouse testis (AP+GSCs), and uncovered the role of IGF-1/IGF-1R signaling in germ cell pluripotency. Interestingly, while comparing with the normoxia (21 % O2), the AP+GSCs showed in a dramatically increase of alkaline phosphatase activity (AP), cell proliferation, HIF-1α, HIF-2α, Oct-4 protein expression, and pluripotent gene expression (such as Oct-4, Sox2, Nanog, Klf-4, and c-Myc) under hypoxia condition (5 % O2). Moreover, the IGF-1 and IGF-1R expression were also significantly increased under hypoxia. Further analysis by using PPP (a specific inhibitor of IGF-1R phosphorylation) and/or LY294002 (a specific PI3K inhibitor) treatment dramatically reduced the Oct-4 expression as well as HIF-1??/HIF-2???nprotein of AP+GSCs. These results highlight the regulation of Oct-4/HIF-2α expression by IGF-1/IGF-1R signaling. In summary, our results demonstrated an up-stream regulation of stemness (Oct-4, Sox2, Nanog, and Klf-4) and tumor factor (c-Myc) of AP+GSCs which was cross-talking with IGF-1/IGF-1R signaling in stem cell transformation under hypoxia condition.