HYPOXIA

HIF1 in opposition

Constitutive expression of the hypoxia inducible factor 1 (HIF1) complex is most often associated with tumour progression and poor clinical outcome. However, expression of HIF1 is also evident during tumour suppression, and new results indicate that this might be the result of HIF1 regulation of the microRNA gene *mir-210*.

CORBIS

Several papers have indicated that *mir-210* expression is induced by hypoxia and that it might be a HIF target gene. To investigate further, Xin Huang, Amato Giaccia and colleagues used a microarray to identify microRNAs that had higher expression in hypoxic than normoxic pancreatic cancer cell lines. Although several microRNAs were induced, *mir-210* had the greatest increase. Further analyses showed that *mir-210* is a HIF1 target in both normoxic and hypoxic cells and has a hypoxia-responsive element in its promoter region to which HIF1α, but not HIF2α, binds.

MicroRNAs can regulate the expression of many mRNAs, and although one can use computer programmes to identify candidate mRNAs through sequence matches between the microRNA and the 3′ untranslated region of the mRNA, this method is not exact and will identify non-physiological target mRNAs. To enrich for potential miR-210-regulated mRNAs, the authors initially used ribonucleoprotein immunoprecipitation in MCF10A cells, which have a robust induction of *mir-210* in hypoxic conditions. These cells were stably transfected with a MYC-tagged argonaute 2 protein — an essential component of the protein complex through which microRNAs regulate mRNAs. Immunoprecipitation of MYC-tagged argonaute 2 in these cells under hypoxic and normoxic conditions and subsequent microarray analyses of the associated microRNAs identified 246 mRNAs that were abundant in the ribonucleoprotein immunoprecipitations carried out under hypoxic conditions. The authors compared these mRNAs with likely miR-210-regulated mRNAs identified using four independent computer programmes. To verify the mRNAs as miR-210 targets using luciferase assays 4 mRNAs from the resulting list of 50 were chosen at random. Two of these genes, homeobox A1 (*HOXA1*) and *HOXA9*, as well as fibroblast growth factor receptor-like 1 (*FGFRL1*), which has multiple miR-210 binding sites, proved to be miR-210 targets.

Surprisingly, overexpression of *mir-210* in pancreatic and head and neck cancer cell lines did not promote growth *in vivo*, but delayed the growth of these cells when they were injected subcutaneously into nude mice. Although expression of *HOXA1* or *FGFR1* mRNAs lacking their 3′ untranslated region negated some of the effect of *mir-210* expression, tumour growth was not restored to the rate shown by the parental lines.

So, expression of *mir-210* is regulated by HIF1α, and in turn miR-210 alters the expression of several genes, some of which seem to be required for the initial growth phase of xenografted cancer cell lines. This work indicates that HIF1 alters two distinct pathways during hypoxia — the well-established pathway that promotes the development of a new blood supply and alters cellular metabolism, and a new pathway that might restrict the initial phases of tumour development. Undoubtedly, more work is needed to understand this tumour-suppressive effect of HIF1.

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ORIGINAL RESEARCH PAPER Huang, X. *et al.* Hypoxia-inducible mir-210 regulates normoxic gene expression involved in tumour initiation. *Mol. Cell* **35**, 856–867 (2009)