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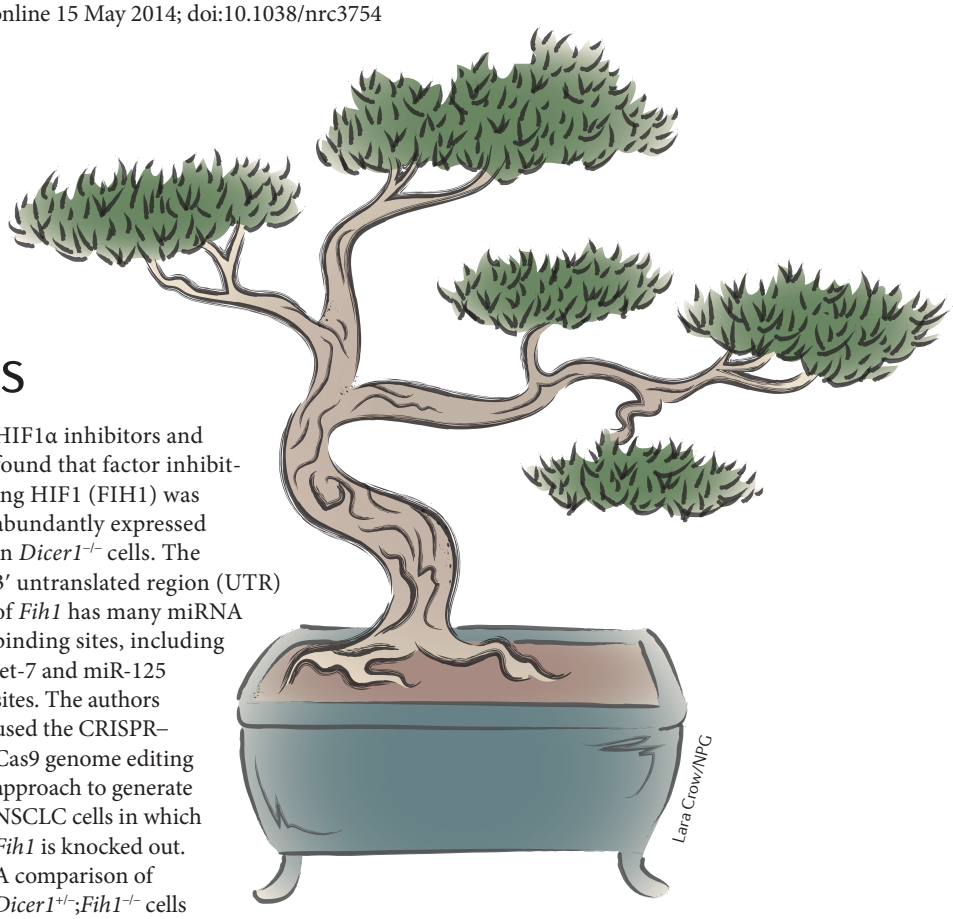

Lara Crow/NPG

HYPOXIA

Micro changes

MicroRNAs (miRNAs) have important and complex roles in tumorigenesis. Sidi Chen, Yuan Xue and colleagues have found that a global loss of miRNAs reduces the proliferation of non-small-cell lung cancer (NSCLC) cells *in vivo* owing to the suppression of tumour angiogenesis.

Despite miRNAs having both positive and negative effects on tumour angiogenesis, the authors found that mouse *Kras*$^{G12D+/-}$;*Trp53*$^{-/-}$ NSCLC cells in which *Dicer1* (a component of the miRNA maturation machinery) was knocked out resulted in tumours in immunosuppressed mice that had substantial areas of hypoxia compared with *Dicer1*$^{+/-}$ NSCLC cells in which mature miRNAs were expressed at normal levels. The authors expected to see increased levels of angiogenesis in the *Dicer1*$^{-/-}$ tumours but, instead, angiogenesis was impaired. mRNA sequencing and pathway analyses indicated that the global loss of mature miRNAs resulted in a reduced expression of mRNAs from genes that are regulated by hypoxia-inducible factor 1α (HIF1α). miRNAs are known to suppress their target mRNAs, which suggests that the downregulation of genes bound by HIF1α is not a direct result of the loss of mature miRNAs and that loss of *Dicer1* must be having an indirect effect on tumour angiogenesis.

So, what is the target that is affected by loss of mature miRNAs? The authors looked at known HIF1α inhibitors and found that factor inhibiting HIF1 (FIH1) was abundantly expressed in *Dicer1*$^{-/-}$ cells. The 3′ untranslated region (UTR) of *Fih1* has many miRNA binding sites, including let-7 and miR-125 sites. The authors used the CRISPR–Cas9 genome editing approach to generate NSCLC cells in which *Fih1* is knocked out. A comparison of *Dicer1*$^{+/-}$;*Fih1*$^{-/-}$ cells with *Dicer1*$^{-/-}$;*Fih1*$^{-/-}$ cells showed that knocking out *Fih1* in a *Dicer1*-null background resulted in increased HIF1 transcriptional activity, whereas loss of FIH1 expression in heterozygous *Dicer1* cells had no effect. Moreover, re-expression of FIH1 in *Dicer1*$^{-/-}$;*Fih1*$^{-/-}$ cells suppressed the transcription of HIF1 target genes, and expression of let-7 and miR-125 in *Dicer1*$^{-/-}$;*Fih1*$^{+/+}$ cells increased HIF1-mediated gene expression owing to the targeting of the *Fih1* mRNA. Injection of *Dicer1*$^{-/-}$;*Fih1*$^{-/-}$ NSCLC cells into mice resulted in tumours in which hypoxia levels were reduced compared with *Dicer1*$^{-/-}$ tumours. Mutation of the 3′ UTR of *Fih1* using the CRISPR–Cas9 system produced a cell clone in which almost all miRNA binding sites had been lost. This clone had increased FIH1 expression levels and reduced HIF1 transcriptional activity. All of these findings indicate that the loss of mature miRNAs in NSCLC cells results in increased expression of FIH1 and suppression of HIF1 transcriptional activity.

Previously published data indicate that basal expression levels of FIH1 in cells are low and *Fih1*-knockout mice are viable with no evidence of deregulated HIF1α activity. These findings are consistent with the effect of FIH1 on HIF1α and tumour angiogenesis being evident only in cells in which the miRNA regulation of *Fih1* is lacking. These findings underline the concept that the regulation of the hypoxic response by miRNAs is part of a complex system.

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> “loss of *Dicer1* must be having an indirect effect on tumour angiogenesis”

ORIGINAL RESEARCH PAPER Chen, S. *et al.* Global microRNA depletion suppresses tumor angiogenesis. *Genes Dev.* http://dx.doi.org/10.1101/gad.239681.114 (2014)