Hypoxia promotes stemness and poor prognosis through epigenetic regulation of DICER

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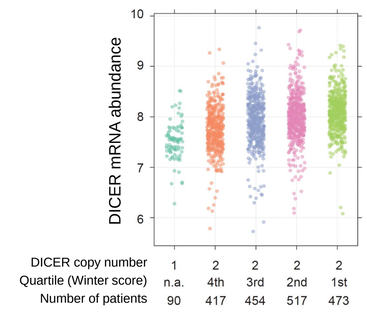
Introduction

MicroRNAs (miRNAs) post-transcriptionally control the expression of a vast number of target mRNAs. Mature miRNAs are generated from longer precursors upon sequential processing by the ribonucleases DROSHA and DICER. Levels of mature miRNA are frequently reduced in cancer, and low expression of DICER in breast, ovarian, and other malignancies is associated with poor clinical outcome. The mechanisms responsible for reduced DICER expression and miRNA biogenesis are not well understood, although monoallelic loss of DICER has been reported in several cancer types. Here, we identify tumor hypoxia as the major regulator of DICER expression in large cohorts of breast cancer patients. We show that DICER expression is suppressed by hypoxia to levels similar to that in tumors with monoallelic loss. We also demonstrate that hypoxia causes silencing of the DICER promoter through a novel epigenetic mechanism requiring the histone 3 lysine 27 (H3K27) methyltransferase EZH2 and inhibition of the oxygen-dependent H3K27me3 demethylases KDM6A/B. Hypoxic suppression of DICER creates a miRNA processing defect and results in selective reduction of mature levels of the miR200 family. Consequently, hypoxia leads to derepression of ZEB1, stimulates the epithelial to mesenchymal transition (EMT), and leads to acquisition of stem cell phenotypes in human mammary epithelial cells. Our study uncovers a previously unknown relationship between oxygen-sensitive epigenetic regulators, miRNA biogenesis and tumor stem cell phenotypes that may underlie the known association of both DICER and hypoxia with outcome in breast cancer.

DICER expression is reduced in hypoxic human breast cancers

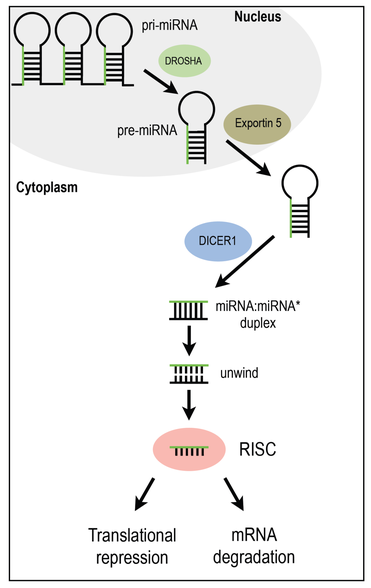


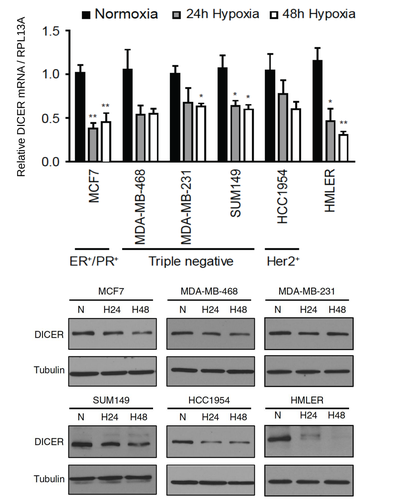
Breast cancer patients from the METABRIC dataset having normal DICER copy number were stratified by the amount of tumor hypoxia as determined using the validated Winter hypoxia signature.

Dataset	Median spearman's correlation	Median spearman's correlation *P*-value
METABRIC	–0.29	1.08E–36
TCGA	–0.24	5.65E–05
Harris	–0.37	3.00E–08
Pooled dataset	–0.23	5.80E–13
Bild	0.12	1.42E–01
Bos	–0.30	1.49E–05
Chin	–0.23	9.57E–03
Desmedt 1	–0.34	1.37E–06
Desmedt 2	–0.12	2.21E–01
Hatzis 1	–0.05	3.39E–01
Hatzis 2	–0.18	1.35E–02
Ivshina	–0.30	1.03E–06
Kao	–0.13	1.60E–02
Miller	–0.29	5.55E–06
Pawitan	–0.45	2.05E–09
Sabatier	–0.37	1.17E–09
Schimdt	–0.28	5.61E–05
Sotiriou	–0.23	2.80E–02
Symmans 1	–0.34	5.75E–03
Symmans 2	–0.15	3.39E–02
Wang	–0.25	1.29E–05
Zhang	–0.13	1.18E–01

Correlation between hypoxia and DICER in individual datasets.

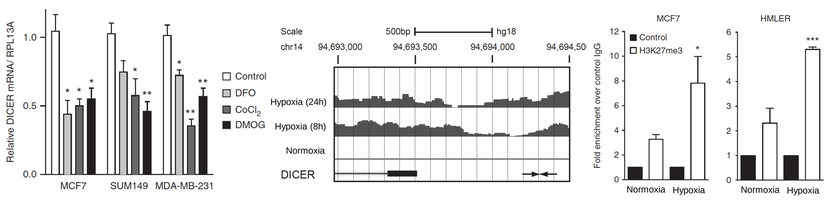
DICER expression is repressed by hypoxia



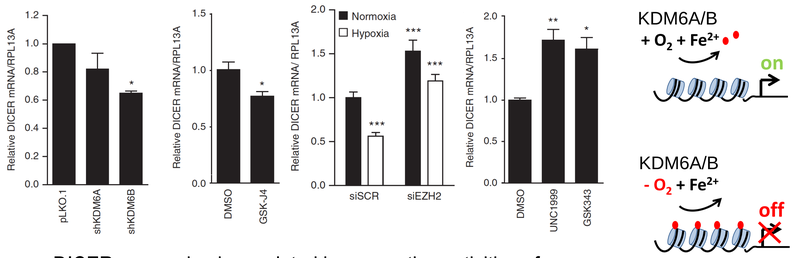


DICER mRNA and protein expression is down-regulated during hypoxia in a panel of breast cancer cell lines.

DICER is epigenetically silenced in response to hypoxia

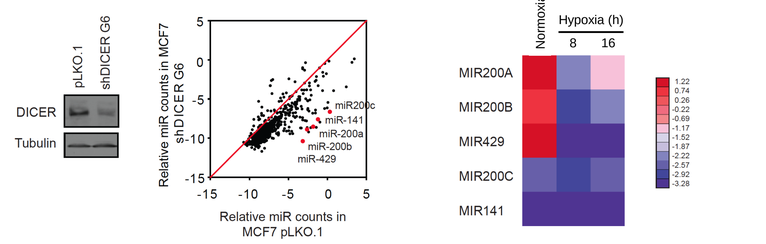


H3K27me3 ChIP-seq demonstrated that hypoxic exposure results in a strong increase in repressive H3K27me3 marks in the DICER promoter region.

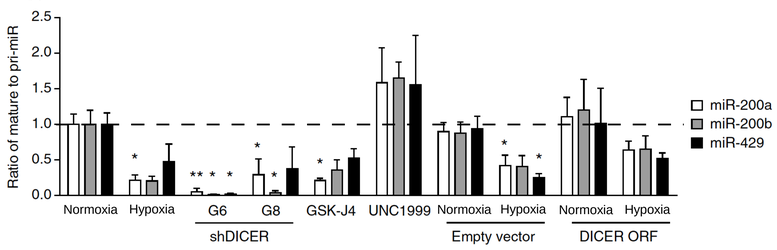


DICER expression is regulated by competing activities of EZH2 and KDM6A/B.

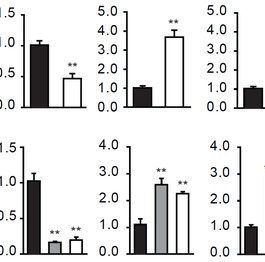
Hypoxia and DICER depletion impairs miR200 processing



Left: Global miRNA levels were assessed using MCF7 cells having vector control or DICER knockdown (shDICER1 G6). Right: Using next-generation sequencing, three of the miR200 family members were identified to be similarly repressed by hypoxia in MCF7 cells.

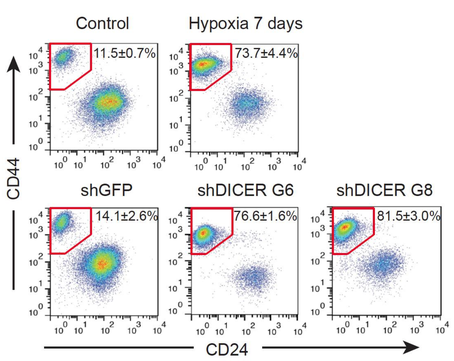


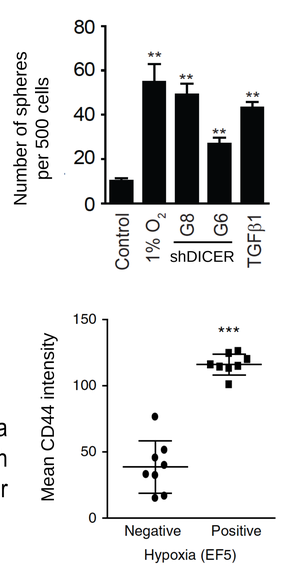
Hypoxia and DICER suppression promotes an epithelial-to-mesenchymal transition



To assess the biological consequences of reduced DICER during hypoxia, human mammary epithelial cells (HMLER) were exposed to hypoxia using the H35 HypOxystation by Don Whitley Scientific.

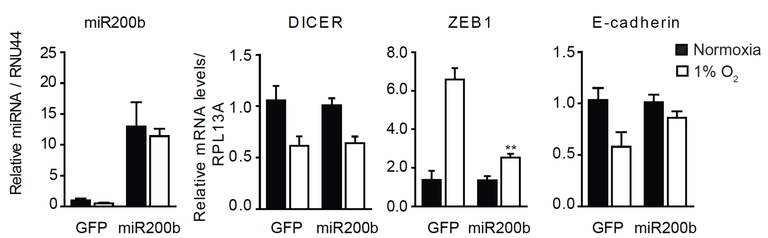
Hypoxia and DICER suppression promotes a stem like phenotype



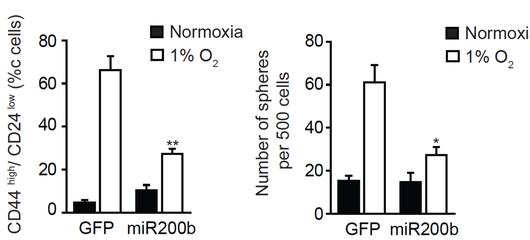


In HMLER orthotopic xenografts, tumor hypoxia as assessed by EF5 is strongly associated with increased expression of the stem cell marker CD44 (n=8 mice).

Hypoxia promotes a stem like phenotype in a miR-200b dependent manner

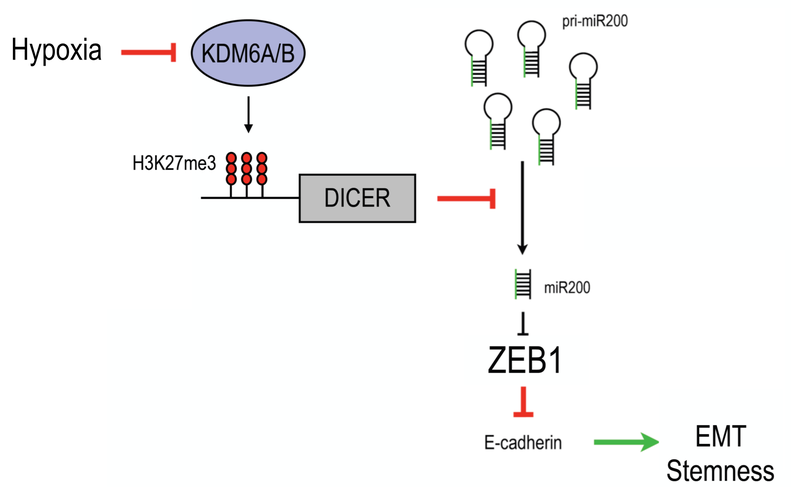


Overexpression of miR200b during hypoxia prevents an epithelial-to-mesenchymal transition (EMT) as assessed by qRT-PCR.



Overexpression of miR200b during hypoxia partially rescues changes in $CD44^{high}CD24^{low}$ cell surface expression and mammosphere formation.

Conclusion



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CIHR IRSC Canadian Institutes of Health Research / Instituts de recherche en santé du Canada
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