HYPOXIA INDUCIBLE FACTOR-1 AND ITS IMPACT ON TUMOR CHEMORESISTANCE IN NEUROBLASTOMA DERIVED CELL LINES

Doktorova H.[1], Hrabeta J.[1], Feglarova T.[1,2], Eckschlager T.[1]

[1]Department of Paediatric Hematology and Oncology, 2nd Faculty of Medicine, Charles University in Prague and University Hospital Motol, Czech Republic;
e-mail: h.doktorova@gmail.com;

[2]Department of Biochemistry, Faculty of Science, Charles University in Prague, Czech Republic

FN MOTOL

INTRODUCTION

The main driving force behind vascularization and neoangiogenesis of solid tumors is tumor hypoxia caused by inadequate blood supply. Hypoxia is one of the reasons for reduced cell death in radiation- and cytostatic-treated cancer cells. Adaptation of tumor cells to hypoxic conditions contributes to the aggressive attitude of tumors and their dedifferentiation. Hypoxia-inducible factor-1 (HIF-1) is the main factor that is necessary for hypoxia adaptation and so targeting the HIF-1 pathway has become an important area for cancer therapy research.

THE AIM OF THE STUDY

HIF-1 is continuously expressed in hypoxia despite an overall decrease in global protein translation. There are also several mechanisms in some types of tumors that regulate the stability and activity of HIF-1 in an oxygen-independent manner. Therefore, in our project, we focus on *in vitro* study of neuroblastoma (NB) derived cell lines and the impact of HIF-1 in normoxia, and in hypoxia induced resistance to commonly used cytostatics which is a major cause of anti-tumor treatment failure in children.

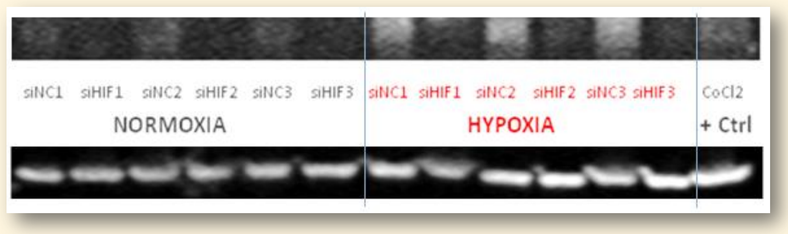
RESULTS

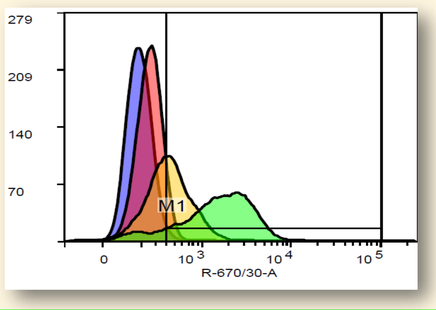
We have found out that using siRNA anti-HIF-1 is responsible for decrease in the amount of viable cells in hypoxia, but also in normoxia. In hypoxia, the inhibition of HIF-1 can suppress the chemoresistance of tumor cells to cisplatin. These experiments are consistent with our previous results, when we used small-molecule HIF-1 inhibitors and shRNA gene silencing.

Verification of HIF-1 silencing

We have used siRNA for HIF-1 knockdown. The effectiveness of silencing was verified by Western blotting and flow cytometry that also displays labeled antibodies.

Western blot for HIF-1 and β-actin in UKF-NB-4 cells after 6,5 hrs in normoxia and hypoxia: siNC – non-coding siRNA (used as control), siHIF – siRNA anti HIF-1. We have used 3 different concentrations of siRNAs (12,5 nM siRNA1, 25 nM siRNA2, 50 nM siRNA3). Cells incubated with $CoCl_2$ were used as a positive control for HIF-1 expression.



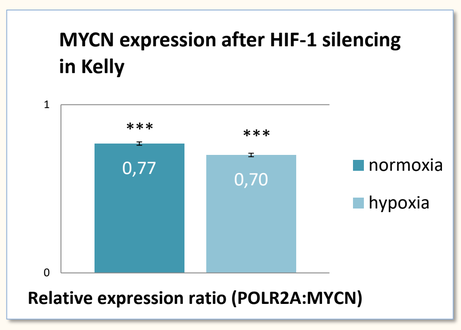


For the ongoing experiments we have chosen the second concentration of previously used siRNA. We have then verified the silencing of HIF-1 in the cell lines (results shown only for Kelly) by flow cytometry.

Color	Population	Event	% Tot.	
	E1 [Ctrl- Kelly siHIF]	5399	53,99	Control reference without antibody
	E1 [Ctrl-_Ab Kelly siHIF]	5387	53,87	Cells with siRNA anti HIF-1 in normoxia
	E1 [Kelly_Ab]	3816	38,16	Cells with non-coding siRNA in normoxia
	E1 [Ctrl+_Ab Kelly hypo]	3408	34,08	Cells with siRNA anti HIF-1 in hypoxia

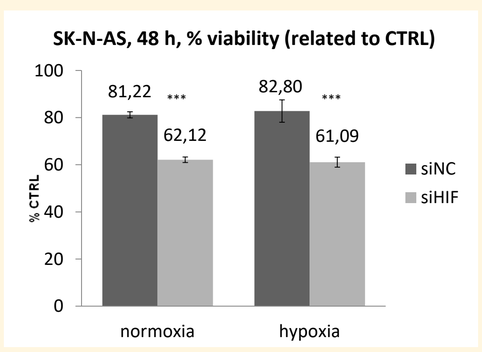
Changes in MYCN expression after HIF-1 silencing

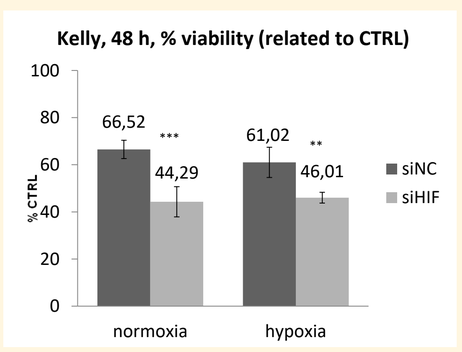
High-risk NB tumors are characterized with MYCN oncogene amplification. It was shown that HIF-1 and MYCN can cooperate in some ways (Qing G. et al.). Due to that fact we wanted to determine if there is a difference in MYCN expression after HIF-1 silencing in the cell lines we´ve observed (Kelly and UKF-NB-4 have the amplification of MYCN). We have performed qPCR for relative quantification of the genes of interest. MYCN expression ratios after HIF-1 silencing in normoxia and hypoxia in Kelly are shown here.

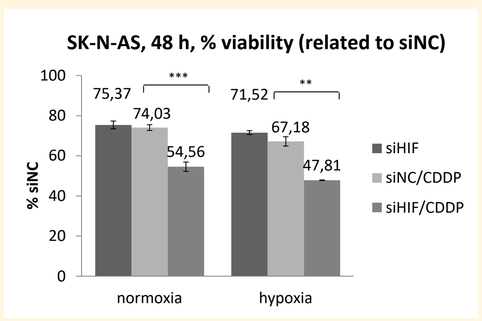


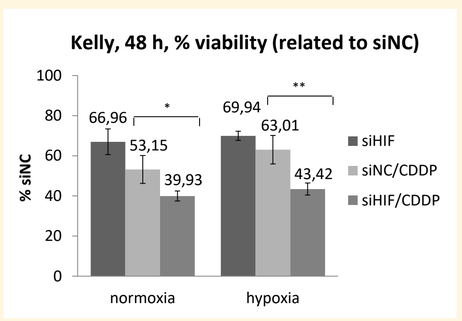
Viability after HIF-1 silencing in normoxia and hypoxia

Based on our observations, we found that the decrease in viability after silencing of HIF-1 occurs independently of MYCN overexpression – a decrease in viability was observed not only in cell lines with MYCN overexpression, but also in cell lines without it.







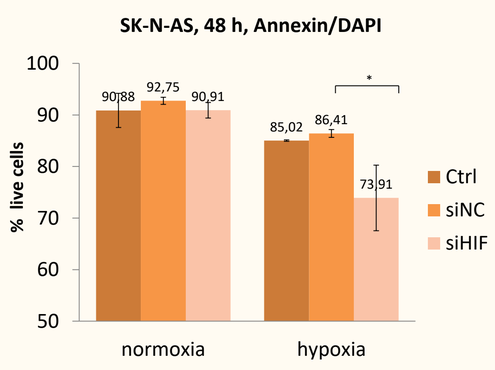


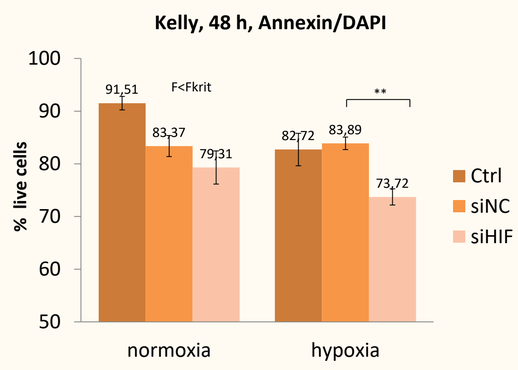
In normoxia as well as in hypoxia, the percentage of viability was significantly reduced after silencing HIF-1 both in cells with and without MYCN overexpression. These observations were made 48 hrs after transfection. The viability is related to untreated cells (CTRL), siNC – cells transfected with non-coding siRNA, siHIF – cells transfected with siRNA anti HIF-1.

Based on our previous study, some NB cell lines show higher resistance to cytostatics in hypoxia (data not shown). Here, we can see the dramatic increase in cisplatine (CDDP) effectiveness after using siRNA anti HIF-1 in both hypoxia and normoxia.

We have also observed apoptotsis via Annexin/DAPI staining (viable cells are shown), 48 hrs after silencing in normoxia or hypoxia (N/H).

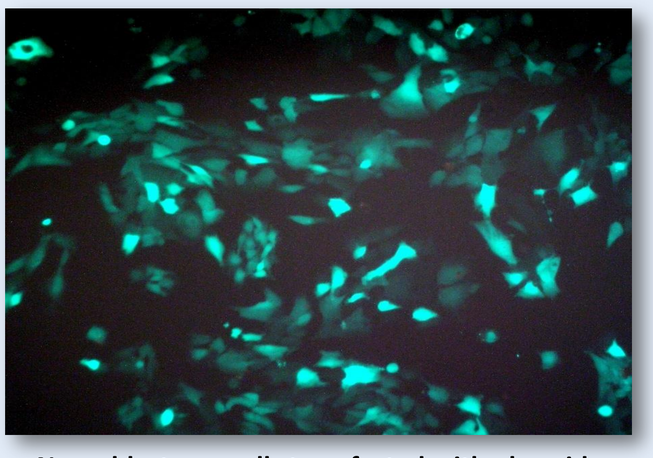
Ctrl – control cells without siRNA in N/H, siNC – cells treated with non-coding siRNA, siHIF – cells treated with siRNA anti-HIF-1.





KEYWORDS

Neuroblastoma, tumor hypoxia, hypoxia-inducible factor, HIF-1α, chemoresistance



Neuroblastoma cells transfected with plasmide coding for GFP – control of transfection (UKF-NB-4 cell line)

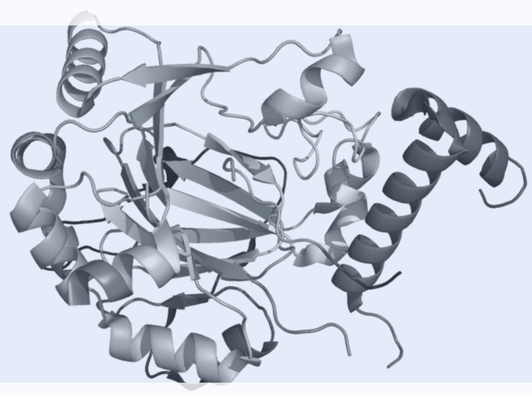
Come and explore HIF-1 acting in tumor cells

MATERIALS AND METHODS

Cells were cultivated in IMDM with 10 % of FBS. Cells in low passages were used (P2 and P3). We have used siRNA anti-HIF-1 in normoxic and hypoxic (1 % O2) conditions in four NB cell lines with (Kelly, UKF-NB-4) or without (SK-N-AS, SH-SY5Y) MYCN amplification. In these cells we performed viability assay after 48 hrs in hypoxia and normoxia with or without cisplatine (CDDP).

CONCLUSIONS

In the past years, HIF-1 has been studied primarily in context with tumor hypoxia. Based not only on our recent results, HIF-1 is involved in tumor agresiveness even in normoxic conditions. Acting of HIF-1 in both normoxic and hypoxic conditions is a very complex field that is still interesting to explore in more detailed way in different types of tumors. Definitely, better understanding of the regulatory mechanisms of HIF-1 will have an impact on the treatment of NB and other malignant tumors.



REFERENCES

Doktorova H., Hrabeta J. et al.: Hypoxia-induced chemoresistance in cancer cells: The role of not only HIF-1. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2015;159(2):166-77

Harris AL.: Hypoxia - a key regulatory factor in tumour growth. Nat Rev Cancer. 2002;2:38–47.

Neuroblastoma in Children. CureSearch for Children's Cancer. Retrieved July 29, 2019, from https://curesearch.org/Neuroblastoma-in-Children

Qing G., Skuli N. et al.: Combinatorial regulation of neuroblastoma tumor progression by N-Myc and hypoxia inducible factor HIF-1alpha. Cancer Res. 2010 Dec 15;70(24):10351-61.

Semenza GL.: HIF-1: upstream and downstream of cancer metabolism. Curr Opin Genet Dev. 2010 Feb; 20(1):51-6. Epub 2009 Nov 26.

Swapan KR.: Editor. Neuroblastoma - Molecular Mechanisms and Therapeutic Interventions, 1st ed. Academic Press, 2019, p. 334.

Westermark UK., Wilhelm M. et al.: The MYCN oncogene and differentiation in neuroblastoma. Review. Seminars in Cancer Biology, 2011; 21:4.