No reason to reconsider HIF-2 as an oncogene in neuroblastoma and other cancer forms

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Westerlund et al. (1) conclude that HIF-2α is linked to neuroblastoma suppression, contradicting several previous observations and current views of the role of HIF-2 in tumorigenesis. We believe that their conclusions are based on flawed cellular models and inappropriate interpretations of their own and public neuroblastoma expression data, as detailed below.

First, HIF proteins are continuously translated but instantly degraded at oxygenated conditions, leading to weak correlation between mRNA and protein levels (2, 3). Given this general mechanism of HIF activation, it is surprising that Westerlund et al. challenge existing models of HIF-2 without examining tumor specimens at the cellular level or HIF-2α expression at the protein level. By analyzing total tumor EPAS1 mRNA, the authors fail to address the fact that most EPAS1/HIF-2α is likely derived from nontumorous cells such as tumor-associated macrophages (4). Supporting this notion and likely explaining the Westerlund et al. data, an established set of macrophage expression markers (5) correlated positively with EPAS1 mRNA levels and favorable neuroblastoma disease in patient cohorts analyzed by Westerlund et al. (Fig. 1). It is unlikely that their findings are related to neuroblastoma cell HIF-2 action. Notably, prior characterizations of HIF-2α in neuroblastoma showed expression in a minority of immature perivascular tumor cells, with the bulk of tumor cells lacking expression (6, 7).

The in vitro models in this study are based on classical neuroblastoma cell lines treated with 5-aza-deoxycytidine (AZA) and retinoic acid (RA), that is, a general DNA demethylator and a hormone with no known biological function in sympathetic nervous system (SNS) development. The authors do not define the cellular phenotype induced, but conclude that global AZA demethylation and RA induce neuronal differentiation. This conclusion is based on morphology and Tuj-1 expression in cultured cells, and on SCG5 and STMN2 expression in tumors from treated mice. Remarkably, none of these markers is specific to the SNS neuronal lineage or neuroblastoma. Analysis of RNA-sequencing (RNAseq) data from their paper reveals, however, that clinically fundamental neuroblastoma SNS markers TH, CHGA, and SYP are not significantly induced (Fig. 2). Thus, AZA+RA treatment does not induce a sympathetic neuronal phenotype in the three cell lines used, in line with previous reports on RA effects on SNS marker gene expression (8).

That HIF-2 has tumor-suppressive rather than tumor-promoting roles in neuroblastoma, an SNS-derived tumor, is controversial and unsupported by previous observations. First, EPAS1 knockout in mice results in impaired development of the SNS rather than, for example, hyperplasia (9). Furthermore, EPAS1/HIF-2α is transiently expressed in developing human and murine SNS (9). Second, in the closely related SNS tumors paraganglioma and pheochromocytoma, gain-of-function mutations in EPAS1 are frequent both as sporadic and inherited events (10). No loss-of-function mutations of EPAS1 in any tumor form have been reported, including VHL-deleted tumor forms like clear-cell renal cell carcinoma.

Given the results presented above of the extended analysis of data in Westerlund et al., and the uniform conclusions of published reports, we see no reason to change the general view that HIF-2 has oncogenic function(s) in neuroblastoma and other tumor forms.

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Fig. 1. Macrophage marker expression correlates with EPAS1 expression and prognosis in neuroblastoma patients. RNAseq data from the 498-patient cohort described in Westerlund et al. was used for all analyses. (A) Scatter plot of EPAS1 expression versus a z-score–based macrophage signature score. (B and C) Overall survival (B) and event-free survival (C) Kaplan–Meier plots divided based on macrophage signature scores. Pearson’s correlation coefficient, $r$, and Bonferroni-corrected $P$ values are displayed where applicable.

Fig. 2. Neuronal differentiation markers are unaltered by AZA+RA treatment. RNAseq data published by Westerlund et al. (GSE100568) on xenograft tumors treated with combinations of RA and AZA were used to examine classic clinical markers of neuronal differentiation in neuroblastoma. Reads per kilobase of transcript per million mapped reads (RPKM) expression values of three clinically used diagnostic neuroblastoma markers of neuronal differentiation (TH, CHGA, and SYP) were displayed according to experimental treatments in three neuroblastoma cell line-derived xenografts (CHP-212, LAN-1, and SK-N-AS; A–C, respectively). ANOVA $P$ values are displayed.