CHAPTER 16

EFFECT OF HYPOXIA ON THE TUMOR PHENOTYPE: THE NEUROBLASTOMA AND BREAST CANCER MODELS

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Abstract: The tumor oxygenation status associates with aggressive behavior. Oxygen shortage, hypoxia, is a major driving force behind tumor vascularization, and hypoxia enhances mutational rate, metastatic spread, and resistance to radiation and chemotherapy. We recently discovered that hypoxia promotes dedifferentiation of neuroblastoma and breast carcinoma cells and development of stem cell-like features. In both these tumor forms there is a correlation between low differentiation stage and poor outcome, and we conclude that the dedifferentiating effect of lowered oxygen adds to the aggressive phenotype induced by hypoxia. With neuroblastoma and breast carcinoma as human tumor model systems, we have addressed questions related to hypoxia-induced molecular mechanisms governing malignant behavior of tumor cells, with emphasis on differentiation and growth control. By global gene expression analyses we are currently screening for gene products exclusively expressed or modified in hypoxic cells with the aim to use them as targets for treatment.

1. INTRODUCTION

Cancer is a multigenetic disease, and the tumor origin determines, at least in part, which genes become affected. In total, some 100 different genes have been shown to be somatically deranged as a consequence of the selective pressure acting on tumor cells [1]. Even within a specific subgroup of tumors there is a considerable genetic variability. This is one explanation of the tremendous

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phenotypic heterogeneity seen in major tumor forms such as breast, colon, and prostate cancer and major pediatric cancers such as neuroblastoma. In the clinical setting, the complexity of cancer cell behavior has become even more evident during the last decades with the realization that not only tumor cells proper determine tumor aggressiveness. Our expanded knowledge of the pathophysiology of tumors shows that the interplay between the genetically unstable and altered tumor cells and the diploid, genetically stable stromal and blood vessel cells most profoundly determines the behavior of tumors and patient outcome. It is well established that tumor stroma affects the metastatic process and is involved in raised interstitial tumor pressure [2], and neovascularization with recruitment of vascular endothelial cells is a necessary and limiting factor in growth of solid tumors [3].

The tumor oxygenation status is tightly linked to aggressive behavior, in part explained by the facts that hypoxia is the major driving force behind tumor vascularization [4,5], and that hypoxia enhances the development of other hallmarks of aggressive tumor phenotypes [6], e.g., high mutational rate [7], metastatic spread [8], lowered pH, and resistance to treatment by radiation or cytotoxic drugs [9]. It is well established that growth of a solid tumor over a size of a few millimeter requires vascularization of the tumor. Tumor hypoxia and expression of vascular endothelial growth factor (VEGF) by stabilization and activation of the hypoxia inducible transcription factors (HIFs) are major players in the formation of new blood vessels, and consequently VEGF signaling and HIF activity are targets for novel treatment strategies aiming at blocking the angiogenic process, and thus tumor growth [5]. In addition, we recently discovered that low tumor oxygen levels lead to reduced expression of differentiation lineage specific genes and to the development of stem cell-like phenotypes, as demonstrated in neuroblastoma and breast cancer [10,11]. We conclude that this effect of hypoxia has a direct bearing on tumor aggressiveness, as tumors with immature features are more aggressive than corresponding, differentiated tumors, which is particularly true in the case of neuroblastoma and breast cancer [12–14]. Thus, in order to understand central issues like phenotypic heterogeneity, metastatic growth, angiogenesis, drug sensibility, and drug resistance, tumor physiological phenomena such as hypoxia, low pH, and high interstitial pressure has to be taken into account.

2. THE CELLULAR RESPONSE TO HYPOXIA

2.1. Hypoxia-inducible factors

All organisms from bacteria to humans have mechanisms for maintaining O_2 homeostasis in order to survive. A low availability of oxygen, hypoxia, results in cellular responses which in vertebrates improves oxygenation and viability through induction of angiogenesis, increase in glycolytic metabolism to raise energy production and upregulation of genes involved in cell survival/apoptosis

[5]. As we know today, the most important proteins governing the adaptive responses to hypoxia in mammals are the hypoxia inducible factors. HIF is a heterodimer consisting of an α-subunit (HIF-1α, HIF-2α, or HIF-3α), which is stabilized and activated with decreasing oxygen levels, and an oxygen-independent β-subunit also known as aryl hydrocarbon receptor nuclear translocator (ARNT) [15]. Both subunits contain basic helix-loop-helix (bHLH) and PAS domains (an acronym referring to the PER, ARNT, and SIM proteins, in which this motif first was discovered) mediating DNA binding and protein heterodimerization. Under hypoxic conditions (frequently 1% O_2 in experimental systems), the HIF-1α subunit translocates within minutes, independently of ARNT, to the nucleus by nuclear localization signals allowing rapid transcriptional responses to lowered intracellular oxygen levels [16,17]. Upon reoxygenation, HIF-1α protein is rapidly reduced with a half-life of <5 min and usually becomes undetectable under normoxic conditions. HIF-1/ARNT heterodimers bind to specific DNA sequences termed "hypoxia response elements" (HREs) present in promoters or enhancers of HIF target genes [18]. Importantly, binding of HIF to HREs and subsequent activation of genes depends not only on the amount of HIF complex, but also on coactivators such as CBP/p300 and posttranslational modifications [19,20].

Of the HIF proteins, HIF-1α was first described and has also been most studied. Two other proteins, HIF-2α and HIF-3α, were later discovered and share several characteristics with HIF-1α such as stabilization under hypoxia, heterodimerization with ARNT, DNA binding, and gene transactivation. Of the two proteins, HIF-2α, which was first named endothelial PAS protein (EPAS-1), has the highest similarity with HIF-1α [21]. HIF-2α is expressed in a complementary but not overlapping pattern to HIF-1α under systemic hypoxia. HIF-3α also shares considerable homology with HIF-1α and HIF-2α, but lacks the C-terminal transactivation domain and could thus act as a suppressor of the HIF pathway [22]. Recently, a dominant negative regulator of the HIF-α subunits named inhibitory PAS (IPAS) domain protein was also identified as a splice variant of the HIF-3α locus [23,24]. Moreover, discovery of sequence homologues of ARNT [25] that may have distinct physiological roles together with the HIF-α proteins further complicates the HIF network and the hypoxic signaling response.

Both HIF-1α and HIF-2α are essential for normal development. *HIF-1α*$^{-/-}$ mice exhibit gross morphological aberrations, and die at approximately embryonal day 11 (E11) due to neural tube defects and cardiovascular malformations, reflecting the importance of HIF-1α during development [26,27]. The greater severity of the *HIF-1α*$^{-/-}$ embryonic defects compared with ARNT-deficient mice [28] indicates that HIF-1α dimerizes with other partners such as ARNT2 [25]. Deficiency of HIF-2α has a more restricted effect on development. During embryogenesis HIF-2α is mainly expressed in vascular structures, but also in different parts of the developing sympathetic nervous system (SNS), responsible for catecholamine production prior to birth. *HIF-2α*$^{-/-}$ mice also die at

E9.5–E13.5 of heart failure as a result of deficient catecholamine production, or of severe vascular defects [21,29]. However, the *HIF-2α*$^{-/-}$ phenotype appears to strongly depend on the genetic background, since defective catecholamine synthesis is not exhibited in all *HIF-2α* knockout strains [29,30].

Adaptation to hypoxia is critical also for cancer cells, implicating involvement of HIF proteins in tumorigenesis. Mouse hepatoma cell lines with mutated ARNT form much smaller tumors that express only low levels of VEGF and do not become highly vascularized [31]. Several studies have also associated HIF-1α with human cancer progression [32,33]. Histological analyses have shown that increased levels of HIF-1α correlate with poor prognosis and resistance to therapy in various solid tumors [34,35]. Overexpression of HIF-1α has also been associated with cell proliferation in several major tumor types, such as colon, breast, lung, skin, ovarian, prostate, and renal carcinomas [35]. The increased HIF-1α activity in cancers can result from intratumoral hypoxia as well as being a consequence of genetic alterations (i.e., by oncogene activation or tumor suppressor inactivation) or stimulation by growth factors [5]. In either case, higher HIF-1α activity leads to upregulation of genes involved in many aspects of cancer progression, including metabolic adaptation, resistance to apoptosis, increased angiogenesis, and metastasis. HIF-2α expression is also increased in a variety of human tumors, including neuroblastomas as will be further discussed in this contribution, but has mostly been associated with stromal cells [34,36]. Compared with bona fide cancer cells, stromal cells might mediate a different response to hypoxia.

2.2. Regulation of HIF stability and function

At hypoxia the HIFs are stabilized due to inhibited protein degradation, whereas at full access to oxygen the HIFs are degraded by the proteasomal pathway. The prolyl hydroxylases (PHD) are key regulators of HIF-stabilization. Using oxygen as substrate, PHDs hydroxylate conserved proline residues in the oxygen-dependent degradation domains (ODD) of the HIF-α subunits [37,38]. The abundance of PHD proteins and their affinities for oxygen are main regulators of the capacity of the PHDs to hydroxylate and thus promote degradation of HIFs. Hydroxylated prolines are recognized by the von Hippel-Lindau tumor suppressor protein (pVHL), which associates with the ubiquitin E3-ligase and forms a complex with HIFs resulting in proteasomal HIF degradation [39]. There are three identified PHDs that recognize HIF; PHD1, PHD2, and PHD3. PHD2 and PHD3 mRNA have been shown to be upregulated in many cell systems at hypoxia, also in neuroblastoma cells (to be published). The accumulation of PHD proteins at hypoxia is part of a negative feedback loop, which is responsible for enhanced degradation of the HIF-α subunits after reoxygenation. Cells transfected with siRNA against the PHDs show prolonged HIF-α stability after reoxygenation [40,41]. Furthermore, PHD2 seems to have a preference for HIF-1α, while PHD3 appears to target HIF-2α for hydroxylation

[42,43]. Both *PHD2* and *PHD3* are known to be HIF-1α target genes, which supports the idea of a negative feedback loop [44,45]. However, our data show a fast and robust, but transient, induction of HIF-1α protein levels at hypoxia (1% O_2) in neuroblastoma cells, whereas HIF-2α reveals a prolonged protein induction pattern at hypoxia [46,47]. The fact that HIF-1α drives the transcription of PHD2 and PHD3 at hypoxia indicates that the HIF-1α negative feedback loop affects HIF-1α protein levels also at low oxygen levels. Studies using siRNA further revealed that PHDs retain their functional activity at oxygen conditions as low as 2% oxygen, indicating a role for PHDs in HIF-regulation at low oxygen pressures [48]. In neuroblastoma cells we have shown that HIF-2α mRNA levels, but not HIF-1α mRNA levels increase at prolonged (72 h) hypoxia [49] and Uchida et al. [50] have seen similar patterns in lung epithelial cells. In summary, our current view is that the mechanisms by which HIF-α proteins accumulate differ between HIF-1α and HIF-2α. At hypoxia the PHDs possibly exert their effect primarily on HIF-1α and not HIF-2α, which could be an explanation for persistent HIF-2α protein levels at prolonged hypoxia in conjunction with increased HIF-2α mRNA levels.

In addition to the PHDs, an oxygen-dependent asparagyl hydroxylase factor inhibiting HIF (FIH) is involved in the regulation of HIF-α transcriptional activity. Under normoxic conditions, FIH hydroxylates an asparagine residue located in the C-terminal activation domain (CAD) of HIF-α. This post-translational modification, which is inhibited by hypoxia, reduces interaction with the CBP/p300 coactivators and results in a decreased ability of HIFs to transactivate their target genes [51].

3. HYPOXIA DEDIFFERENTIATES TUMOR CELLS

3.1. The neuroblastoma model

Neuroblastoma is a childhood tumor derived from immature SNS or SNS precursor cells, and based on differentiation marker gene expression analysis most neuroblastomas express a neuronal phenotype [52]. However, a subset of neuroblastomas contains tumor cells that have undergone a spontaneous neuronal-to-neuroendocrine/chromaffin lineage shift in areas adjacent to zones of tumor necrosis [52,53]. With the discovery of the HIFs, and in particular HIF-2α that is selectively expressed in the developing SNS, including neuroendocrine cells [10,21], we hypothesized that the differentiation lineage shift seen in these neuroblastomas might be hypoxia- and HIF-2α–driven. We did indeed detect HIF-2α and HIF-1α protein in these neuroendocrinely differentiated tumors in the zones of tumor cells surrounding necrotic areas, albeit HIF-2α protein was abundant also in tumor cells close to blood vessels, and thus presumably well oxygenized tumour areas [46]. However, when we experimentally addressed the question of whether hypoxia promotes neuroendocrine differentiation using a panel of human neuroblastoma cell lines grown under hypoxic conditions, our

data suggested that hypoxic neuroblastoma cells, rather than adopting a neuroendocrine phenotype, lost their differentiated characteristics. We arrived at that conclusion based on expression analysis of neuronal differentiation markers, which became downregulated at the same time as HIF-2α and HIF-1α proteins were stabilized [10]. Thus, growth at low oxygen pressure (1% O_2) resulted in decreased expression of neuronal/neuroendocrine marker genes (*Chromogranin A* and *B*, *NPY*, *GAP43*, *dHAND*, and *HASH-1*) and neurofilament (*NEF3*), while genes linked to the decision of neural crest cells to develop into a sympathetic precursor cell (*KIT*, *ID2*, *NOTCH1*, and *HES1*) were upregulated [10,46,49]. Global gene expression analysis of hypoxic neuroblastoma cells confirmed these data and highlighted a number of additional genes supporting our conclusion that hypoxia drives neuroblastoma cells towards an immature phenotype [49].

As neuroblastomas are derived from the SNS, which in turn stems from the neural crest [54], we concluded that hypoxic neuroblastoma cells gain stem cell-like neural crest characteristics. Hedborg et al. have addressed the same question and also analyzed the hypoxic phenotype of cultured neuroblastoma cells [55]. They came to the conclusion that hypoxic neuroblastoma cells in culture go through a neuronal-to-neuroendocrine transition, similar to the in vivo situation in some neuroblastomas. However, their conclusions are based on a limited number of cell lines and marker genes, and importantly, the only established neuroendocrine marker investigated, *chromogranin A* (except for two bona fide hypoxia-driven genes, *IGF-2* and *TH* [10,56,57]) did not increase in their hypoxic cells. Thus, our and Hedborg's data are largely in agreement, but we come to different conclusions. As our conclusion is based on a substantial number of cell lines, on global gene expression data, and analyses of several recognized neuronal and neuroendocrine markers, we are confident that human neuroblastoma cells of established cell lines do not differentiate toward a neuroendocrine phenotype at hypoxic (1% O_2) growth conditions. Recently, it was reported that the nerve growth factor receptor TrkA exists in a truncated, NGF-independent, constitutively activated form in neural stem cells and in some neuroblastomas [58]. Treatment of neuroblastoma cells with the hypoxia mimetic cobalt chloride induced the expression of the truncated, stem cell-associated form of TrkA, supporting our findings that hypoxia pushes neuroblastoma cells to a stem cell-like phenotype, although these authors never directly tested the effect of hypoxia.

Clearly, cultured hypoxic neuroblastoma cells do not differentiate into a neuroendocrine lineage, and their response to hypoxia do not reiterate the neuronal-to-neuroendocrine lineage shift seen in a subset of clinical neuroblastomas. In retrospect, this finding is perhaps not so surprising as most neuroblastoma specimens do not show this lineage shift [12,52]. In general, neuroblastoma cell lines are established from highly aggressive, immature tumors, while those neuroblastomas showing neuroendocrine features appear to be more differentiated. Therefore, one might argue that established neuroblastoma cell lines most likely do not represent neuroblastoma cells with a capacity to differentiate into

a neuroendocrine lineage. From a clinical as well as a tumor biological perspective, the dedifferentiating effect of hypoxia on tumor cells, highlighted by the study of high stage neuroblastomas, is probably a far more important observation.

3.2. The breast carcinoma model

The effect of hypoxia on tumor cell differentiation is not restricted to neuroblastoma and experimental tumor models, which we demonstrated in ductal breast carcinoma in situ [11]. In these tumor lesions, hypoxic cells, surrounding the necrotic zones and expressing HIF-1α protein, are morphologically immature by standard clinical histopathological criteria compared with well-oxygenized cells close to the basal membrane layer surrounding the tumor duct. Interestingly, hypoxic tumor cells had high expression of the breast epithelial stem cell marker, cytokeratin 19, and in estrogen receptor (ER) positive tumor lesions, the ER expression was downregulated in hypoxic cells ([11], Kronblad et al., to be published), presumably as part of a hypoxia-induced dedifferentiation process. We postulate that hypoxia-induced tumor cell dedifferentiation is one mechanism by which hypoxia contributes to the selection of a malignant tumor phenotype, as poor differentiation correlates positively to adverse outcome in both breast cancer and neuroblastoma [12–14].

4. MOLECULAR MECHANISMS OF HYPOXIA-INDUCED DEDIFFERENTIATION

The sequential molecular steps involved in the conversion of migrating neural crest cells to sympathetic precursors and finally to differentiated nonmigrating sympathetic neuroblasts, or SNS chromaffin cells, are far from known. However, genes shown to be important for lineage determination of SNS precursor cells were found to be affected by hypoxic treatment in neuroblastoma [10]. Hypoxia downregulated members of the bHLH transcription factor family, such as the neuronal markers *HASH-1* and *dHAND* while their counteracting HLH factors, the ID proteins, became upregulated. Furthermore, the dimerization partner for HASH-1 and dHAND, the E-protein E2-2 was also downregulated by hypoxia [49]. As also *NOTCH-1* and its downstream-regulated genes, including *HES-1*, were activated by hypoxia, a causal link between the NOTCH/HES pathway and *HASH-1* downregulation might exist and contribute to the dedifferentiated phenotype [10,49]. These results taken together suggest a contributing molecular mechanism by which hypoxic neuroblastoma cells dedifferentiate (Figure 16.1). A more direct involvement of HIF proteins in this process achieved further support when we demonstrated that hypoxic *ID2* expression was regulated by HIF-1 [59]. In that study the expression of another ID member, *ID1*, was also increased by hypoxia. The hypoxia-induced ID proteins could play a significant role in the initial phase of dedifferentiation by sequestering E-proteins such as

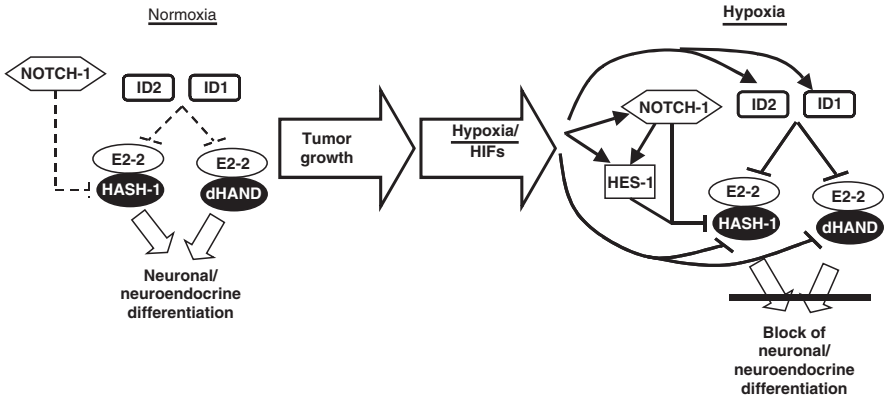

Figure 16.1. Molecular mechanisms involved in dedifferentiation of hypoxic neuroblastoma cells. Under normoxic conditions, the ID proteins are expressed at moderate levels allowing functionality of E-proteins (E2-2) in complex with tissue-specific bHLH proteins (i.e., dHAND and HASH-1) leading to expression of neuronal marker genes. In expanding solid tumors, regions of intratumoral hypoxia are formed where hypoxia-inducible factors (HIFs) are stabilized and functional. In the hypoxic microenvironment, the tissue-specific transcription factors and their dimerization partners are downregulated with a concomitant HIF-induced expression of *ID2* (and potentially *ID1*). These processes together with a hypoxia-induced activation of the NOTCH/HES signaling network result in downregulation of neuronal and neuroendocrine markers and development of a less differentiated phenotype. Based on results published in [10,49,59].

E2-2, and thereby inhibiting dimerization with HASH-1 and dHAND and their DNA-binding capacities. Reduced expression of neuronal bHLH transcription factors, together with inhibited bHLH function by ID proteins as a response to hypoxia would have profound effects on genes regulated by HASH-1 and dHAND. The fact that *ID* genes are downregulated upon induced differentiation in neuroblastoma [60], and are reduced during differentiation of several other cell types [61,62], further implicates their role in hypoxic dedifferentiation of neuroblastoma and likely other tumors as well. Since upregulation of *ID1* and *ID2* also was found in hypoxic breast cancer cells [59], and cells expressing HIF-1α in lesions of ductal breast carcinoma in situ were morphologically dedifferentiated [11], a possible connection between the ID proteins and breast tissue-specific bHLH factors may exist and be affected by hypoxia.

The involvement of HIF proteins, directly or indirectly, in hypoxic gene transcription of differentiation-related genes has not yet been investigated on a full scale in neuroblastoma or in other tumor types. It is likely, although not certain, that genes rapidly induced by hypoxia are direct HIF-target genes since HIF-1α and HIF-2α proteins become instantaneously stabilized and active in response to lowered oxygen in almost all cell types, including neuroblastoma [10,16].

5. CLINICAL HETEROGENEITY AND STABILITY OF THE HYPOXIC PHENOTYPE

Most solid tumor forms show extensive phenotypic heterogeneity both among tumors of the same diagnosis group and within a given tumor, as revealed by histopathological examination. As hypoxia has profound effects on the phenotype of any cell type including tumor cells, oxygen shortage contributes substantially to tumor heterogeneity. The hypoxic response is similar in most cells, i.e., increased expression of classical hypoxia/HIF-driven genes like *VEGF*, *GLUT-1* and *GLUT-3* and genes coding for glycolytic enzymes. Microarray analysis based on 27,000 genes and ESTs of seven different human neuroblastoma cell lines grown at 21 or 1% O_2, revealed an overall, uniform hypoxic response with induction of well-established hypoxia-driven genes like those mentioned above (Fredlund, Ovenberger, and Påhlman, to be published). A more detailed analysis disclosed a considerably more complex picture. While only four genes where up- or down-regulated twofold or more in all seven cell lines, the expression of as many as 7,000 genes were changed twofold or more in at least one of the seven cell lines. We could not show that the response in one or a few cell lines stood out against the others, which would offer an explanation why so few genes were uniformly regulated in all tested cell lines. Instead we have data showing that the discrepancies are explained by an unpredictable lack of or reduced hypoxic response of individual genes in one or two cell lines and that these cell lines differ from one gene to another. For example, in one neuroblastoma cell line the glucose transporter gene *GLUT-1* was downregulated when *GLUT-3* was upregulated by hypoxia. In another cell line, the opposite pattern was seen, while in the remaining five cell lines both *GLUT* genes were upregulated as expected [63], since both genes have been shown to be HIF-1α-driven [5]. Thus, for unknown reasons, the hypoxic response in at least human neuroblastoma cells is not as coherent as one might have expected, and translated to the clinical situation, adverse responses to low oxygen levels of subsets of tumor cells within a given tumor, will add to the complexity of the tumor phenotype.

In a growing tumor, the oxygenation level is not static but changes with the formation of new, and collapse of old tumor blood vessels. We therefore tested how persistent the immature features of hypoxic neuroblastoma cells are upon reoxygenation, as this will reflect the situation of an extravasating cancer cell encountering well-oxygenated blood. Expression analysis of a selection of early neural crest markers as well as later sympathetic markers revealed that the dedifferentiated hypoxic neuroblastoma phenotype persisted for up to 24 h. This was the situation irrespective of whether hypoxic neuroblastoma cells were reoxygenated to an atmospheric (21% O_2) and not very physiological condition, or to a more physiological tissue oxygen pressure (5%). However, the hypoxic phenotype was reversible after 72 h of reoxygenation (Figure 16.2). Intermittent (cycles of 1 and 21% O_2), as well as long-term (12 days) hypoxia reinforced the

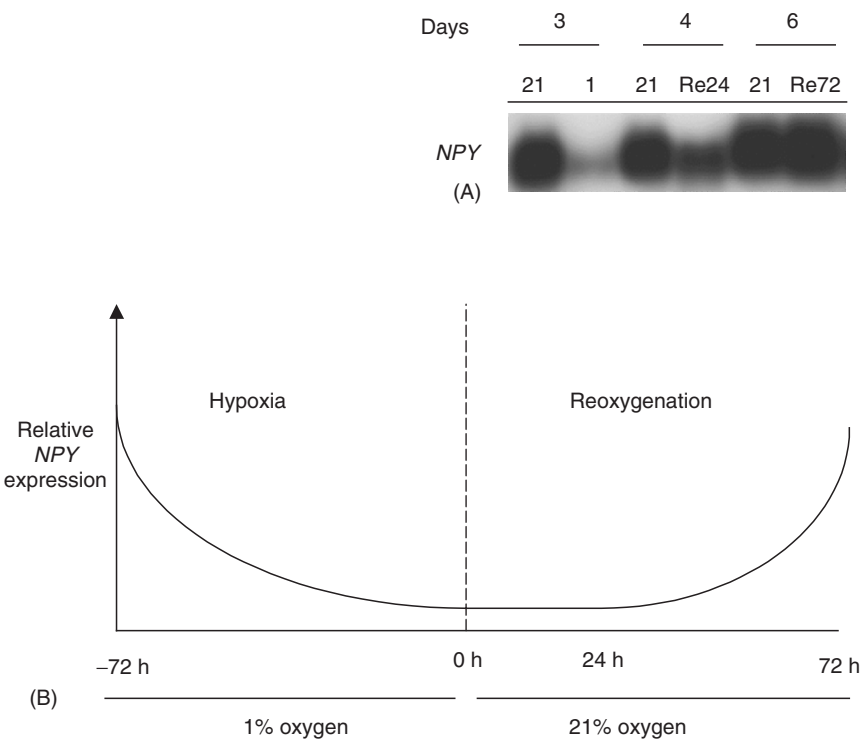

Figure 16.2. Phenotypic changes in reoxygenated (Re) hypoxic neuroblastoma cells, as exemplified by the expression of the neuronal marker, *neuropeptide Y* (*NPY*). (A) Northern blot analysis of *NPY* in KCN-69n human neuroblastoma cells grown at 1% O_2 (1) for 3 days followed by reoxygenation at 21% O_2 (21) for 24 or 72 h. (B) Graph illustrating the relative expression of *NPY* (and other neuronal marker genes) in reoxygenated neuroblastoma cells. Adapted from [47].

hypoxic phenotype but it was still reversible after 72 h of reoxygenation at 21% O_2 [47]. In agreement with the findings of Hedborg et al., we conclude that the hypoxic response is reversible upon reoxygenation.

6. TARGETING THE HYPOXIC PHENOTYPE

The hypoxic phenotype is principally tumor-specific and one overriding aim of our ongoing hypoxia project is to identify genes that are strongly induced by hypoxia (and hypoglycemia) and to use these genes as targets for treatment. We are specifically focusing on genes that are coding for cell surface proteins. An ideal candidate gene would have limited expression in normoxic tumor cells, as well as in normal tissues. Given the dedifferentiating effect of hypoxia on tumor cells, we are currently searching our microarray databases for hypoxia-inducible

genes normally expressed early during development, with initial focus on growth factor receptors, membrane channels, and adhesion molecules. As an alternative strategy, as well as an important step to verify that changes in gene expression identified by microarray analysis are indeed correlating to altered protein levels, protein fractions from normoxic and hypoxic tumor cells are analyzed by 2-D gel electrophoresis followed by mass spectroscopy identification of hypoxia-induced proteins or protein modifications induced by hypoxia. If we identify proteins that are more or less uniquely expressed or modified in hypoxic cells, the idea is to generate antibodies and to test their selectivity for hypoxic tumor cells both in vitro and in animal tumor models.

7. CONCLUSIONS

The observation that hypoxia appears to be a general mechanism by which tumors develop an immature phenotype is conceptually novel and highlights a new aspect of the aggressiveness of hypoxic tumors. The hypoxic phenotype, being essentially tumor-specific, is an attractive target for treatment. As outlined here, uniquely or highly overexpressed genes/proteins in hypoxic cells might successfully be explored as antibody targets, and might target the tumor stem cell compartment generally believed to be resistant to treatment.

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