The Molecular Mechanism of HIF-2α-Mediated ADRN-MES Phenotypic Transition Under Hypoxia in Neuroblastoma Cells

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Abstract. Neuroblastomas are usually located in an area with poor oxygen supply inside the tumour; such an environment may promote metastasis and other serious behaviour in children. Tumor cells can switch between adrenergic and mesenchymal states to affect the clinical outcomes of young children.Hypoxia causes changes in the shape of cancer cells, the whole molecular signal pathway is still not known, and essential regulators that control these switches at different stages of cell growth have not been identified, Therefore,many crucial regulatory circuits still need to be studied. Based on the data of hypoxic cell lines, genetic knockdown assays and various molecular diagnostic methods were used to identify the metabolic pathways that cause structural changes in neuroblastoma under oxygen deprivation. HIF-2α is a switch that changes the expression pattern of the adrenergic signature genes into a mesenchymal one. PRRX1 and NOTCH3 are the secondary mediators that can decrease the production of native adrenergic mRNAs and promote the activation of a pro-tumorigenic mesenchymal feedback loop via HIF-2α. Currently, all of them are still at the level of laboratory cell culture and have not been validated by living mouse experiments or histopathological tests of real human tumour tissue. In the future, new technologies and a new biological model of rodents will be introduced into clinical trials to further verify the application of this model and enhance the development of HIF-2α-targeted drugs. This research provides a clear molecular basis for follow-up experimental exploration.

Keywords: Neuroblastoma, Hypoxia, Cytoplasmic Redistribution, HIF-2α.

1. Introduction

Neuroblastoma (NB) is a non-blood tumor of the newborn that develops from neural crest cells in the adrenal glands or thoracic sympathetic ganglia, typically appearing in early childhood. There are various clinical signs of the disease [1]. Low-risk patients have been doing well in the recovery process after surgery and chemotherapy for a long time; at the same time, children in the high-risk group are more likely to develop serious metastasis, drug resistance and recurrence, and their five-year survival rate is more than 50 per cent. Therefore, it is a serious problem in the field of children's cancer treatment.

A state of hypoxia can promote the development of malignant tumours and is thus considered one of the essential features in the development process of many aggressive tumours [2]. Rapid and disorderly growth of tumor cells can damage the structure of blood vessels, obstruct normal blood flow in the vessels, reduce the supply of essential oxygen to the surrounding area, etc. Therefore, there would be changes in the metabolic requirements and structure of the organism. Based on the experimental results, many hypoxic niches have been found in histological samples of neuroblastoma; due to the lack of sufficient oxygen, they are closely associated with the spread, mobility and sensitivity to radiotherapy of this disease and function as an important factor in carcinogenesis. Such hypoxic features lay the foundation for studying tumor cell phenotype shift.[3]

Current research shows that there are two separate neuroblastoma cell populations at present, and their statuses change back and forth between the mesenchymal (MES) subtype and the adrenergic (ADRN) subtype. The two types of cell phenotypes have different genomic structures and biological functions: MES cells have upregulated components of the NOTCH pathway, maintain strong migratory ability and aggressiveness, promote tumour invasion and spread, are resistant to therapy, and on the other hand, ADRN cells increase the level of tyrosine hydroxylase (TH) and dopamine β-

hydroxylase (DBH) significantly and are sensitive to mitogenic inhibition; thus, they are the main oncogenic drivers. The division into these particular tissue sections helps us understand some basic ways neuroblastoma can go into remission, evade the immune system, or be resistant to radiation and drugs. Only the malignant state is removed in medical therapy; oxygen-deprived-induced phenotypic plasticity has been ignored and is now known to be one of the reasons for the reduced effectiveness of modern treatment.It is necessary to clarify the full regulatory pathway of this phenotypic change.

Now, many studies have been carried out to understand the phenotypes of different subsets of neuroblastoma cells and build basic theories; single-cell technology has precisely identified the genomic blueprints of MES and ADRN categories, shown that the NOTCH pathway is a primary control mechanism maintaining mesenchymal characteristics, and through combined laboratory experiments and histological murine models, it has been found that in a low-oxygen environment, there is an urgent structural change from ADRN entities to a MES-like structure.More evidence is required to complete the whole regulatory framework of hypoxia response.

HIF-1α is required to maintain metabolic activity in the absence of oxygen, thus it plays various roles in ischaemia; it can remain in the cytoplasm before entering the nucleus to regulate gene expression at many locations and activate several stress-responsive genes [4]. Although a large number of studies have found that hypoxia is associated with HIF-1α and NOTCH, not all of the changes in cells under adverse environments are connected; at the same time, the various patterns of histone methylation are also relatively unexplored, and thus a comprehensive and systematic understanding of the initial stage of hypoxia is still missing. The complete linkage between hypoxia and related gene networks requires systematic clarification

Anti-ADRA1D monotherapy alone in oncology can only reduce the size of the tumour temporarily, and at the same time, changes in MESK2 due to haemolysis have been severely resistant to conventional chemotherapy; thus, it may be one of the causes of post-operative recurrence. To control the differentiation of cells under hypoxia, Elucidation of energy metabolism patterns in hypoxic cells is essential; thus, a universal strategy can be established to suppress the proliferation of rapidly dividing cells and strengthen their defense mechanisms, offering novel therapeutic directions for neuroblastoma treatment. Revealing hypoxic metabolic rules helps design combined anti-tumor therapies.

The following are all the molecular mechanisms that regulate the stiffness of neuroblastoma cells in hypoxia. The first idea in this paper is that the change in paediatric solid tumours' architecture is due to oxygen deprivation; at present, the regulatory network mediating phenotypic switching remains poorly characterized, and thus the scientific basis for understanding the plasticity of paediatric cancer cells remains incomplete.This paper systematically sorts out the relevant molecular regulatory mechanisms.

2. Regulatory Role of the HIF Pathway in Hypoxia Response and Cell Phenotype Switching of Neuroblastoma

Hypoxia-Inducible Factors (HIFs) are important transcription factors that respond to low-oxygen conditions inside the cell and then change the expression of many genes. A lack of oxygen in the small area where many cancer cells have grown and problems with blood vessel structure in solid tumours is a typical characteristic. In the normal atmosphere, proline hydroxylase domain-containing enzymes (PHDs) hydroxylate HIF-α; at this time, it is recognized by the Von Hippel-Lindau tumor-suppressor protein complex and is rapidly degraded by the proteasome. On the contrary, it is still in the HIF framework under hypoxia. With a decrease in partial pressure, the activity of PHD decreases; thus, HIF-α does not need to be degraded in the cytoplasm and can form a complex with HIF-1β to promote the transcription of some target genes. HIF has started to change the traits of cells in oxygen-depleted areas and is now known as a sensitive oxygen sensor.This oxygen-sensing system dominates all cell adaptation behaviors under hypoxia.

Neuroblastoma (NB) has shown significant clinical differences from HIF-2α compared with HIF-1α, and it is one of the factors that can distinguish NB from various other solid tumours. Based on the clinical data of many countries, an increased level of HIF-2α is associated with a poor prognosis, widespread spread of systemic metastatic disease and stem-like characteristics in NB patients; at the same time, HIF-1α often appears in the early stages of carcinogenesis or when there is an amplification of MYCN [5]. Therefore, different biological functions may be exhibited by the various HIF family members at different times in the development of NB. HIF-2α can promote tumour angiogenesis, increase stemness and enhance the metastatic ability of normal B-cells (NB) through regulation of its target VEGF and CD55, Thus, it is considered one of the necessary oncogenic factors in NB. HIF-2α is a strong transcriptional activator of many more genes than the other hypoxia-responsive elements, and it also regulates cell fate in the presence of oxidative stress; therefore, it has offered a new research direction for the evolution of NB phenotypes under hypoxia. The precise duration of HIF-2α stability and the specific stage of phenotypic switching where this regulatory event takes place remain uncharacterized; the regulatory effects of relevant factors (promotion or inhibition) also require further exploration. There are still many unsolved problems, such as the reciprocal regulation of HIF-2α and HIF-1α, and their practical differences in several subtypes of NB.Distinct functions of HIF family members determine tumor progression differences.

3. Molecular Pathways of HIF-2α in the Transition from Adrenergic to Mesenchymal Phenotype

Neuroblastoma (NB) has two types of cells with different characteristics: the developed adrenergic (ADRN) group and the MES subtype that has progenitor-like features. There is a high concentration of dopaminergic markers, such as tyrosine hydroxylase (TH) and dopamine beta-hydroxylase (DBH), in ADRN cells, and they have a complicated neuronal structure. At the same time, the MES variant is also a NOTCH pathway component and has shown an increase in migratory ability, chemoresistance and invasive stem cell characteristics. Many important regulation networks (CNs) have kept a balance. The transcription factor PHOX2B and ASCL1 in the ADRN lineage are part of a two-way feedback loop with the genes that control the expression patterns of adrenergic genes. CNs are in contact with NOTCH signalling in the MES state, but it is not known whether there are particular transcription factors involved or what kind of molecular mechanism promotes such a change.Two cell subtypes rely on independent circuits to maintain stable cell characteristics.

Even when deprived of oxygen, HIF-2α can still move to the nucleus and upregulate the expression of genes involved in the mesenchymal phenotype, such as PRRX1 and NOTCH3 [6]. Therefore, it can be concluded that there is a particular molecular link between hypoxia-induced signals and changes in cell structure. In the past, it has been found that PRRX1 is required for the function of HIF-2α, and an increase in the amount of PRRX1 can trigger mesenchymal transformation of ADRN cells independently of other upstream factors. At the same time, an increase in NOTCH3 also promotes the mRNA expression of genes involved in mesenchymal development and may initiate a strong positive feedback loop to strengthen its own function and prevent the recurrence of oncogenic mutations in the original ADRN state. HIF-2α can promote the transcription of PRRX1 and NOTCH3, and at the same time regulate the necessary regulatory network of ADRN to form an independent mesenchyme-biased transcriptional network that increases the stability, spread and clinical significance of tumor stem cells. As shown in the biochemistry circuit of NB cells above, when deprived of oxygen, there is a rapid change in their protein profile due to HIF-2α and a switch from an adenocarcinoma type to a mesenchymal type. However, the exact composition of the CRC MES-type transcriptional regulator is not yet known, and many subtle characteristics of the transient transitional interval during the switch from ADRN to MES are also unknown. Some investigators have put forward that there is a sequence of staged transformations in this intermediate stage, but contradictory evidence shows that the characteristics of the two different conditions are slightly different.Further cell and animal tests are needed to confirm the real transformation process.

4. Mechanisms for the Maintenance of the Mesenchymal Phenotype and the Reversibility of its Conversion

Epigenetic signatures triggered by hypoxia will still be present after the oxygen levels have risen again. Therefore, it is proposed that such biochemical changes and complex transcriptional rearrangements will be very stable and are not merely temporary biological fluctuations. According to the results of single-cell analysis, the classifications of ADRN and MES may change; however, their development paths differ, and although the transition from ADRN to MES usually happens relatively quickly, specific cell metamorphosis takes several days after ischemic injury, and the reverse process of MES to ADRN is not very fast. Even after the hypoxic trigger or HIF-2α antagonist is withdrawn, cells with MES characteristics will not quickly switch back to the ADRN state [7]. Asymmetry is regarded as one of the changes in the MES stage, and after its formation, a stronger feedback loop and molecular modifications (such as DNA methylation, histone modification, and alteration of chromatin structure) are triggered to bolster the resistance of the cell to all sorts of damage.The stable memory of MES state is the key cause of tumor chemotherapy failure

Academic discussion on the basic pillars of communicative functions between ADRN and MES is still lacking at home and abroad. Recently, researchers from oncology and molecular developmental biology have been exploring the transformation processes of ADRN in combination with MES phenotypes in neuroblastoma, and based on the information obtained through single-cell sequencing technology, two different theoretical frameworks have been developed.The two hypotheses form two opposing views on cell transformation patterns.

4.1. The Hypothesis of "Continuous Spectrum Transition"

Many studies have found that there is no clear division among these stages, and thus, neoplastic cells start to show continuous changes in ADRN and MES molecular signatures under the effect of dual selection pressure in reality. A lot of detailed single-cell multi-omics analyses have been conducted on high-grade neuroblastoma, and in 2025, it was reported by Nature Genetics that researchers used single-cell transcriptomic sequencing of clinical samples before and after drug treatment. Four main groups of cells were obtained by means of hierarchical clustering; among them, ADRN and one group mainly expressed MES traits, and frequently a bridging group that is an integrated combination of ADRN-like and MES-like labels also appeared. According to the pseudotime trajectory model, this transient cell population is positioned between the ADRN and MES states; therefore, it has various gene expression patterns, and it can be concluded that the state transition is a lineage-specific evolutionary path rather than an artifact caused by the merger of multiple cell types.Continuous intermediate cell groups are regarded as the transitional form of phenotype switching.

4.2. The Hypothesis of "Mutually Exclusive Discrete States"

At first, some studies found different transcriptomes for the ADRN and MES subtypes; However, with the help of new sequencing technology and modular analysis, it was discovered that they were analytical artifacts and not actual intermediates. Around 5,300 single cells were obtained from the periphery of neuroblastoma in 2022, and then they were analysed by Smart-seq2. After removing the technical noise and extraneous records, most of the segregated cells do not show the same behaviour when tagged with ADRN- or MES-. According to the investigation, many of the previously recognised "integrative signatures" are in fact false positives caused by the simultaneous isolation of ADRN and MES pairs in droplet partitioning, or they have been increased by the randomness of sequencing. There is no genuine transitional stage with different phenotypes; the two groups are entirely separate and autonomous. This is probably due to NB heterogeneity, and because different pathological samples, cell cultures or specific histological areas have different frequencies of such phenomena. Existing results from many laboratories are still inconsistent and have not confirmed or refuted the hypothesis; at present, it is not known whether a transitional state exists [8]. At present,

research has been carried out on the combination of spatial profiling, lineage-specific markers and functional cell assays to understand how different environments affect the development of neuroblastoma cells and what reasons for such changes exist.More multidimensional verification experiments are required to judge which hypothesis is correct.

5. Unresolved Key Scientific Problems and Future Directions of Translation

Although previous studies have shown that HIF-2α promotes the metaplastic change of ADRN cells towards the MES state [9], many important questions have yet to be answered. First and foremost, the specific transcriptional machinery that is engaged in response to HIF-2α is still not well-known, and its complex genomic connectivity pattern in neuroblastoma has not yet been mapped by ChIP-seq. As both PRRX1 and NOTCH3 are now under the regulation of HIF-2α, other unknown adjacent factors may also change the cell's phenotype, and an organised hierarchical control network of these genes has not been formed yet. At present, the experiments do not have specific time information for the changes in the structure of the body at this time [8]. Although single-cell studies have put forward the idea of a "continuum spectrum" of development, this has not been verified by any official method. Finally, there is no clinical trial. Most of the data come from laboratory cell lines and mouse models; thus, the various characteristics of primary tumours obtained from patients and their correlations with treatment outcomes have not been conclusively proven, and conceptual progress has failed to be realised in clinical care.The gap between basic laboratory findings and clinical application needs to be filled.

Based on the main problems mentioned above, future studies by scholars should focus on solving these specific issues. Together with single-cell multi-omics and spatial transcriptomics, CRISPR screening can help us better understand changes in cell structure and networks and discover new biomarkers at all stages of life and diseases, etc. Some preliminary and extensive experiments have been carried out in the medical field to find small-molecule inhibitors of HIF-2α for neuroblastoma; new ways to overcome pharmacological tolerance and address the problem of metastasis may be found through these studies [10, 11]. Through gene analysis, the present research identifies many abnormal oncogenes in the hypoxic area; therefore, can develop personalised inhibition plans to suppress the growth and drug resistance of high-grade malignant tumours, and at the same time, a new treatment path can be planned by targeting the transcription factor group that includes HIF-2α and other MES-dependent factors to improve the clinical effect of treating neuroblastoma.

6. Conclusion

Recently, researchers in many countries and organisations have been studying the changes in stiffness of neuroblastoma cells under hypoxia, and at the same time, many crucial regulatory mechanisms triggered by a lack of oxygen have been identified so far. Previously, it was found that HIF-2α supports the sympathetic structural framework and promotes the production of mesenchymal molecules together with PRRX1 and NOTCH3. Therefore, it may have a malignant nature and be resistant to chemotherapy due to stem-cell characteristics. Many studies have shown that the mechanical memory characteristics of MES under hypoxia are persistent; thus, it can be assumed that these physical changes are associated with all kinds of genetic modifications and epigenetic modifications.

By means of spatial multi-omics investigation at the single-cell level, this study investigates the relationships among all kinds of cell components in malignant hypoxic niches, and based on the above results, preclinical models and human histological tissues have been analysed to suggests that HIF-2α inhibitors can treat invasive neuroblastoma and reduce problems such as metastasis and clinical resistance.This work offers new targets and ideas for clinical intervention of neuroblastoma.

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