**[Cross talk of HIF1α/YB-1 in promotes metastasis of Ewing sarcoma](http://www.bm-jcc.net/cellularofcancer/183)**

**Abstract**

Ewing sarcoma (ES) is a part of a larger family of round blue-cell tumors and a large body of literature exists on ES of bone. Highly metastatic cancers most often strike teens and can be found in younger children and adults. Recent research has added numerous new proteins that can direct the metastatic process either directly or indirectly by inducing a type of protein leading to death in cancer patients. Among them, Y-box–binding protein-1 (YB-1) is a recently identified protein known to induce metastasis in different cancers. YB-1 is mainly involved in the regulation of mRNA transcription, splicing, translation, and stability. However, the molecular mechanisms underlying these biological behaviors have not been completely elucidated. In this study, we investigate the molecular mechanism by which YB-1 promotes HIF1α-induced invasion and metastasis of Ewing sarcoma.

Ewing sarcoma, Metastasis, YB-1

**Introduction**

Malignant growth is described from one viewpoint by sporadic intracellular cycles, and then again by deviant extracellular cycles like a changed interaction between dangerous cells and the cancer microenvironment (TME) [1]. One of the critical parts of the TME is hypoxia, which is by and large characterized as need might have arisen for ordinary cell capability [2]. Hypoxia will foster in most strong cancers as a result of expanded cell expansion and oxygen need as well as of lacking vessel development and blood supply [3]. Concentrates on recognized the hypoxia inducible element (HIF) protein family as key record factors that start the cell transformation to hypoxia [4]. To go about as a record factor, the constitutively communicated subunit HIF-1-b and one of the three oxygen-conditionally communicated subunits HIF-1-a/HIF-2-a/HIF-3-a must dimerize and tie to hypoxia reaction components (HREs) in the objective quality groupings [5]. In this manner, HIFs direct a large number of practical pathways that can influence growth movement, for example, growth vascularization by means of vascular endothelial development factor (VEGF) [6], growth digestion through solute transporter family 2 part 1 (SLC2A1, also called Overabundance 1) [7] and Aldolase-C articulation [9], and cancer motility and obtrusiveness through loss of E-cadherin and initiation of Wnt/beta-catenin flagging [8].

Notwithstanding, the job of HIFs in malignant growth cells goes past intervening the reaction to hypoxia: Truth be told, HIF-1-a can be upregulated through development factors or oncogenic flagging fountains, for example, the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/AKT serine/threonine kinase 1 (Akt) and Ras/Raf/mitogen-initiated protein kinase (MAPK) pathway as well as through inactivation of growth silencers like the phosphatase and tensin homolog (PTEN) protein [9]. This enactment of HIF-1-an in normoxia through elective pathways has been called pseudohypoxia [10] and opens another viewpoint on HIF-1-an as an organization center point to coordinate other cell and ecological signs next to hypoxia [11]. Moreover, the administrative components behind HIF-1-b (likewise named aryl hydrocarbon receptor atomic translocator (ARNT)), HIF-1-a's dimerization accomplice, have been proposed to be more intricate, since HIF-1-b levels appeared to be impacted by hypoxia too [12]. Contrarily, there is proof for HIF-free cell reactions to hypoxia, further testing a shortsighted perspective on hypoxia and HIF flagging [13]. Consequently, in this audit, we purposefully don't utilize the terms hypoxia and HIF articulation/flagging conversely however treat the two factors independently. Besides, per definition, the terms normoxia and hypoxia are utilized in this audit as per Hammond et al., wherein normoxia alludes to 21% oxygen strain, which is the environmental oxygen tension and standard cell culture condition, and hypoxia alludes to oxygen levels inadequate to fulfill the need of the comparing tissue [14]. Of note, the purported normoxic oxygen levels don't mirror the physiological oxygen strains of most tissues, which change between 3-7.4% oxygen (frequently alluded to as physoxia [15]).

While the significance of hypoxia in tumorigenesis and movement has been widely considered and explored in various disease types [6], the momentum information and particularities of hypoxia and HIF motioning in Ewing sarcoma (EwS) have not been methodicallly checked on to date. EwS is the second most regular bone-related cancer dominatingly happening in kids, teenagers, and youthful grown-ups [16]. EwS was at first depicted over quite a while back by the American pathologist James Ewing in 1921, yet the exact cell of beginning still needs not entirely settled [7]. Regardless of this histogenetic vulnerability, EwS is hereditarily very much described: In all cases, EwS is driven by illusory record factors encoded by FET::ETS combination oncogenes, most ordinarily Ewing sarcoma breakpoint district 1 protein (EWSR1)::Friend leukemia coordination 1 record factor (FLI1) (EWSR1::FLI1) (85% of cases) [24]. Hypoxia and HIFs are particularly applicable with regards to EwS on the grounds that: I) hypoxia is a basic part of the bone microenvironment assuming a significant part in the advancement of bone growths [6]; ii) there is an immediate interchange between HIF-1-an and EWSR1::FLI1 at the sub-atomic level[17]; iii) there is major areas of strength for an of broad cancer putrefaction (probable brought about by hypoxia) with metastasis and more terrible patient endurance [8].

Subsequently, the points of this survey are to sum up the latest discoveries on hypoxia and HIFs in the EwS setting, and to give a precise soundness of the accessible information on this subject.

**The phenotype of EwS cells under hypoxia and/or HIF-1-a activity**

Several studies in EwS cell lines grown as monolayers (i.e., 2D) yielded controversial results concerning the effect of hypoxia on cellular proliferation [18]. However, Riffle et al*.* showed that in EwS spheroids, oxygen gradients divided cells according to distinct oxygen tension into populations with different proliferative states [[7](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR4)]. Specifically, EwS cells in the spheroid core stained for hypoxia and apoptosis markers but not for proliferation markers. Reversely, cells at the spheroid surface stained for Ki-67, indicating active proliferation, but exhibited neither hypoxia nor apoptosis markers [[9](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR4)]. Most interestingly, cells that resided at the interface between both populations and thus were exposed to moderate hypoxia were positive for Ki-67 staining and activated DNA damage repair (DDR) enzymes [[7](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR4)]. This suggests that cell cycle is compatible with moderate hypoxia but probably dependent on co-activated DDR [[19](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR4)]. In other tumor entities, such as head and neck squamous cell carcinomas, cells that retained proliferative capacity under hypoxia have been associated with lower survival and tumor aggressiveness, highlighting the clinical importance of studying these subpopulations [7]. However, severe hypoxia is not compatible with EwS proliferation [[20](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR4)]. Regarding the influence of HIF-1-a on EwS cell proliferation, two studies conducted in normoxia and 1% oxygen condition showed that *HIF-1-a* silencing reduced proliferation of EwS cell lines in vitro, indicating a proliferation inducing effect of HIF-1-a in normoxia and hypoxia [7]. However, Knowles et al*.* reported that knockdown of either *HIF-1-a*or*HIF-2-a*increased the proliferation of EwS cells under 0.1% oxygen tension, suggesting an anti-proliferative effect of both genes in EwS cells under very severe hypoxic conditions [[2](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR36)]. These discrepancies concerning the influence of *HIF-1-a*/*HIF-2-a* on EwS proliferation could be due to the different oxygen concentrations that were used in the experiments, implying that the influence of *HIF-1-a*/*HIF-2-a* on the EwS cell phenotype depends on the specific degree of hypoxia [7]. Additionally, HIF-1-a levels vary exponentially within the range of hypoxic conditions, probably contributing to the above mentioned discrepancy of findings in EwS cells in hypoxia [5]. In this context, several authors have emphasized the importance of monitoring pericellular oxygen levels and using standardized techniques for hypoxia models in vitro [7]. This could reduce discrepancies in results and help to elucidate on the influence of hypoxia and HIFs on the EwS phenotype and pathophysiology.

**Apoptosis**

Like in the case of cellular proliferation, diverse findings exist for the question on how hypoxia modulates apoptosis of EwS cell lines. Ryland et al*.* suggested that hypoxia does not induce apoptosis in EwS and found the epigenetic repression of the Potassium Voltage-Gated Channel Subfamily A Member 5 (KCNA5) gene to be involved in EwS cell survival under hypoxic stress [[7](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR43)]. Likewise, Kilic et al*.* confirmed reduced apoptosis of EwS cells under hypoxia and argued for a pro-survival role of hypoxia by showing that low oxygen tension protected EwS cells from chemotherapeutic-induced apoptosis [[21](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR34)]. However, other reports provided evidence that hypoxia activated apoptosis in EwS cell lines [[4](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR36)] and that hypoxia and apoptosis markers co-localized in the center of EwS spheroids [[7](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR4)]. In this context, it is intriguing that even studies that used the same cell line (A-673) and identical culture conditions (< 1% oxygen tension) yielded opposing results [20]. On a similar note, the role of HIF-1-a in mediating apoptosis in EwS cells is controversial. Kilic et al*.* proposed that HIF-1-a protected EwS cells from apoptosis under hypoxia, as knockdown of *HIF-1-a* or therapeutic inhibition of the PI3K/Akt pathway that induced HIF-1-a activity, re-established hypoxia-induced apoptosis [7]. In contrast, Knowles et al*.* noted that *HIF-1-a* and *HIF-2-a* were not involved in mediating the increased apoptosis rate that they observed under hypoxia, as knockdown of either gene did not change apoptotic rates [[7](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR36)]. Interestingly, in diverse cancer types and non-cancerous tissues, it has been shown that hypoxia and HIFs can both trigger apoptosis and confer resistance to it [19], which is in agreement with the described contradicting observations on the relationship between hypoxia, HIF-1-a, and apoptosis in EwS. As discussed in the section on proliferation, differentiating between finely adjusted hypoxia and HIF levels within experimental conditions as well as improvement and standardization of techniques could advance our understanding of EwS pathophysiology and possibly elucidate on the discrepancies in study findings up to date [7].

Migration and invasion

Malignant growth is described from one viewpoint by sporadic intracellular cycles, and then again by deviant extracellular cycles like a changed interaction between dangerous cells and the cancer microenvironment (TME) [1]. One of the critical parts of the TME is hypoxia, which is by and large characterized as need might have arisen for ordinary cell capability [2]. Hypoxia will foster in most strong cancers as a result of expanded cell expansion and oxygen need as well as of lacking vessel development and blood supply [3]. Concentrates on recognized the hypoxia inducible element (HIF) protein family as key record factors that start the cell transformation to hypoxia [4]. To go about as a record factor, the constitutively communicated subunit HIF-1-b and one of the three oxygen-conditionally communicated subunits HIF-1-a/HIF-2-a/HIF-3-a must dimerize and tie to hypoxia reaction components (HREs) in the objective quality groupings [5]. In this manner, HIFs direct a large number of practical pathways that can influence growth movement, for example, growth vascularization by means of vascular endothelial development factor (VEGF) [6], growth digestion through solute transporter family 2 part 1 (SLC2A1, also called Overabundance 1) [7] and Aldolase-C articulation [9], and cancer motility and obtrusiveness through loss of E-cadherin and initiation of Wnt/beta-catenin flagging [8].

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According to Aryee et al., hypoxia promoted anchorage-independent growth of EwS cell lines and marginally enhanced their clonogenicity [[22](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR29)]. Interestingly, EwS cells exposed to hypoxia could stimulate sphere formation of non-hypoxic EwS cells in their surrounding [[7](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR35)], which appeared to be mediated by HIF-1-a.

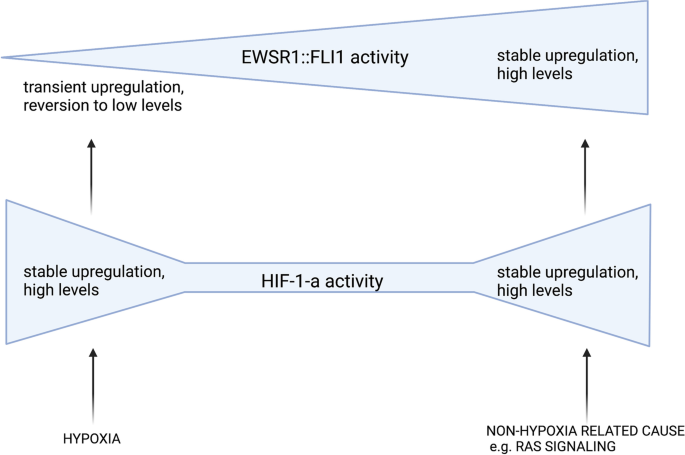
**The role of hypoxia and/or HIF-1-a activity in molecular signaling pathways in EwS**

Intra-cancer heterogeneity is a deep rooted growth trademark that has been applied to the different articulation levels of EWSR1::FLI1 that exist in EwS cells. Evidently, EWSR1::FLI1 articulation is dynamic inside single cells, but the component behind this vacillation isn't perceived [7]. Comparably to EWSR1::FLI1, HIF-1-an articulation has been demonstrated to be heterogenous across EwS cancers and perhaps at the same time inside a given EwS growth [22]. Strangely, in immunohistochemical examination of EwS growths and western smear of EwS cells, HIF-1-a for the most part restricted to the core under normoxia [20], standing out from discoveries in skeletal muscle were HIF-1-a was found just in the cytoplasm in normoxia [23]. Moreover, HIF-1-a co-limited in some yet not all cancer segments with areas of putrefaction. In synopsis, proof exists that HIF-1-a, as EWSR1::FLI1, adds to growth heterogeneity in EwS.

Most strangely, a few reports showed that HIF-1-a heterogeneity and EWSR1::FLI1 heterogeneity could be unthinkingly connected to one another: Aynaud et al. showed that both exceptionally high and extremely low degrees of EWSR1::FLI1 movement were related with decreased EwS cell expansion and upregulation of HIF-1-an objective qualities [7]. Moreover, HIF-1-a straightforwardly incited EWSR1::FLI1 articulation [24]. In this unique situation, we propose an elective view, where HIF-1-a flagging a hypoxia might influence EWSR1::FLI1 articulation levels freely. As a matter of fact, both, hypoxia-subordinate and non-hypoxia-subordinate HIF-1-an enactment have been displayed to prompt EWSR1::FLI1 action in EwS [20]. Nonetheless, for the hypoxia-intervened acceptance of EWSR1::FLI1, Aryee et al. showed that EWSR1::FLI1 protein levels were simply briefly increased and returned to low articulation levels inside 24 h of hypoxia, while HIF-1-an articulation appeared to be steadily instigated. In view of these perceptions, we propose the accompanying situation for the communications of hypoxia, HIF-1-an and EWSR1::FLI1 in EwS: Non-hypoxia-intervened HIF-1-a movement that prompted EWSR1::FLI1 action could depict the component in the cell populace described by high action of the two proteins, HIF-1-an and EWSR1::FLI1. Interestingly, hypoxia-intervened HIF-1-an action that instigates just transient height of EWSR1::FLI1 could be the component portraying the cell populace that is described by high action of HIF-1-an and low EWSR1::FLI1 action [5]. The non-proliferative condition of this cell populace fits in accordance with the idea that solid hypoxia isn't viable with expansion in EwS [11]. Moreover, these cells could be portrayed by the two perceptions that EwS cells with low EWSR1::FLI and EwS cells presented to hypoxia [7] showed expanded transitory and obtrusive potential. In any case, it isn't clear why EwS cells with both high EWSR1::FLI1 and high HIF-1-a movement, are non-proliferative [30], and it very well may be of extraordinary interest to additionally portray this cell populace. One clarification could be that the high HIF-1-a movement itself forestalls EwS multiplication, yet the specific impact of HIF-1-an on Ews expansion isn't obvious to date (see segment on multiplication). Of note, EWSR1::FLI1 prompted by HIF-1-a through hypoxia was most presumably upregulated on a posttranscriptional level [7], while EWSR1::FLI1 that was initiated by HIF-1-an in normoxia by means of Ras flagging was upregulated by means of direct restricting of HIF-1-a to the EWSR1::FLI1 advertiser [2].

By and large, there is proof that hypoxia and HIF-1-an are two key variables adding to the powerful guideline of EWSR1::FLI1 in EwS [7]. In view of the examined reports we recommend that both hypoxia, and HIF-1-a may contribute freely to the guideline of EWSR1::FLI1 (Fig. 1).

**Fig. 1**

[](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w/figures/1)

Hypoxia-related and non hypoxia-related upregulation of HIF-1-a might contribute independently to EWSR1::FLI1 regulation

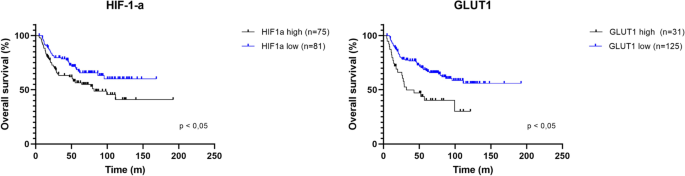
[**Full size image**](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w/figures/1)

**Hypoxia and/or HIF-1-a activity and therapy and resistance in EwS**

Hypoxia and HIFs and prognostic markers in EwS

Therapeutic options to target hypoxia in childhood cancers have been recently reviewed and the urgent need for prognostic markers to evaluate hypoxia in the pediatric setting has been highlighted [[7](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR21)]. Therefore, expression of HIF-1-a, HIF-2-a, and their downstream targets such as VEGF, GLUT1, carbonic anhydrase 9 (CA9), phosphoglycerate kinase 1 (PGK1), and lysyl oxidase (LOX) was evaluated and their association with prognosis and chemotherapy-response seemed to vary between pediatric cancer entities [[7](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR21)]. In this review, we evaluated the correlation of gene expression levels and survival in our cohort of 156 EwS patients and identified high *HIF-1-a* and *GLUT1* expression to be significantly associated with worse prognosis (Fig. [2](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#Fig2)), which was not observed for *PGK1*, *LOX*, *HIF-2-a*, *VEGF*, and *CA9* (not shown). Our results are in line with the notion of Bernauer et al*.*, that i) genes related to HIF signaling could serve as prognostic markers, and ii) the relationship between these genes and survival probably depends on the specific tumor type [[7](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR21)]. Regarding *HIF-1-a* expression at the mRNA level (*n* = 156), our data is in contrast with Knowles et al*.* who did not find a correlation between HIF-1-a expression and survival at the protein level in their EwS patient cohort [[11](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR36)]. Besides the possible difference between the mRNA and protein level, one additional explanation could be that the cohort of Knowles et al*.* cohort was perhaps too small (*n* = 56) to detect a significant difference in survival between HIF-1-a high and low expressing tumors. Most interestingly, HIF-1-a’s downstream effector *GLUT1* was associated to reduced survival in our cohort with very high significance, suggesting GLUT1 as potential biomarker for EwS prognosis (Fig. [2](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#Fig2)).

**Fig. 2**

[](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w/figures/2)

Elevated *HIF-1-a* and *GLUT1* expression correlates with worse overall survival in EwS patients. Kaplan–Meier survival analyses in 156 EwS patients based on *HIF-1-a* and *GLUT1* expression levels (cut-off defined as best percentile, log-rank test). Microarray data were retrieved from the Gene Expression Omnibus (accession codes: GSE63157, GSE34620, GSE12102, GSE17618) and normalized using Robust Multiarray Average (RMA) using custom brainarray chip-description files (v20). Batch effects were removed with ComBat. Tumor purity was assessed using the ESTIMATE algorithm. Only samples with a tumor purity > 60% corresponding to The Cancer Genome Atlas (TCGA) standard were included in survival analyses

**Hypoxia and/or HIF-1-a activity and therapy in EwS**

Targeting hypoxia in EwS treatment has been proposed since more than a decade [7] and corresponding preclinical and clinical studies have been conducted. Preclinically, the Ras inhibitor salirasib reduced EwS growth and migration in vitro and in vivo [[7](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR31)]. Interestingly, salirasib also reduced HIF-1-a and EWSR1::FLI1 protein levels in vivo, suggesting its therapeutic potential in EwS treatment [[5](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR31)]. However, there are no clinical trials for salirasib in pediatric patients so far. Furthermore, melatonin induced hydroxylation and inactivation of HIF-1-a in EwS cell lines, leading to reduced aerobic glycolysis, increased reactive oxygen species (ROS) levels, and apoptosis [[6](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR71)]. Melatonin was well tolerated by pediatric patients in a dose-escalation study [[3](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR72)] and could be a promising candidate for further clinical investigation. Additionally, El Naggar et al*.* found that the class I histone deacetylase (HDAC) inhibitor MS-275, also named etinostat, inhibited YB-1 binding to target gene transcripts and constrained translation of stress-adaptive proteins, among them HIF-1-a [[3](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR73)]. Even though the report focused on NFE2 like bZIP transcription factor 2 (NFE2L2) as mechanistic explanation for the in vivo anti-tumor effects of MS-275 in EwS, the fact that MS-275 also decreased HIF-1-a translation should not be overlooked. MS-275 was well tolerated in the pediatric setting, including EwS patients, and one study reported stable disease for one year under MS-275 treatment in a EwS patient. Further studies are needed to evaluate the therapeutic potential of MS-275 in EwS patients, potentially also in combination treatment. As mentioned above, the role of Src in hypoxic EwS cells is currently discussed and Bailey et al*.* demonstrated that dasatinib, a Src inhibitor, decreased EwS motility and invasion [[7](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR33)]. Yet, two caveats for the use of dasatinib are that i) it seemed not to inhibit proliferation rates in EwS cell lines and ii) the strong rebound effects that have been observed in vitro [[7](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR33)] which suggest that dasatinib should be combined with other drugs for EwS treatment. In line with this, single agent therapy with dasatinib was not efficient in EwS patients in a phase II study [[3](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR76)] and a phase I/II study testing the combination of dasatinib with additional chemotherapeutics in pediatric solid tumors is ongoing (NCT00788125). Furthermore, the CXCR4 signaling axis that is probably linked to hypoxia in EwS has been identified as therapeutic target to reduce EwS migration [[9](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR48)]. In a phase I/II study, the CXCR4 inhibitor plerixafor was well tolerated by pediatric patients, including patients with EwS [[8](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR77)]. However, plerixafor currently is only used as drug to mobilize hematopoietic stem cells from the bone marrow [[2](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR77)] and potential effects on EwS growth and metastasis have not been investigated clinically yet. Of note, bevacizumab, a monoclonal antibody against the HIF-1-a-downstream target VEGF, showed promising anti-tumor effects in combination treatment in EwS in two clinical studies. Additionally, studies in glioma cell lines and patient-derived colon cancer xenografts showed that irinotecan, which is known as DNA damaging anti-cancer agent, can downregulate *HIF-1-a* mRNA and protein levels. This sheds light on the potential mode of action of irinotecan in EwS treatment, where it is already successfully applied. Lastly, geldanamycin, which indirectly inhibits HIF-1-a, was tolerated in a phase I study by pediatric patients, including EwS patients [4]. However, it remains unclear if this drug has anti-tumor efficiency in EwS and further studies are ongoing (NCT00093821). In summary, available preclinical and clinical data support the notion that targeting hypoxia, HIF-1-a, and their associated pathways represent a promising therapeutic strategy in EwS. In this context, drugs targeting hypoxia could be especially useful as an addition to the standard chemotherapeutics in EwS treatment. However, phase II/III studies of hypoxia-targeting drugs in EwS are still missing, and further research in this field is urgently needed.

**Hypoxia and/or HIF-1-a activity and therapy resistance in EwS**

The suggested combination of 4-HPR with safingol [6] could probably improve therapy effectiveness, but still needs to be investigated. As another example, metformin promisingly reduced proliferation of EwS cells and sensitized them to chemotherapeutics in vitro, such as vincristine and doxorubicin [[7](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR91)]. However, in vivo experiments did not show any reduction in tumor proliferation through metformin, neither as single agent therapy nor in combination with other chemotherapeutics [[7](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR91)]. In fact, hypoxia, which was present in vivo but not in vitro, counteracted the anti-proliferative effects of metformin that were observed in vitro [[5](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR91)]. Accordingly, hypoxia had a substantial impact on EwS therapy options implying that more physiological-like cell culture methods in the field of EwS and drug discovery are urgently needed. Most interestingly, Nan et al*.* found that imatinib could reverse hypoxia-induced resistance of EwS cells to metformin, most probably via inhibition of HIF-1-a activity [[4](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR39)]. Hence, concomitant application of metformin and imatinib reduced EwS proliferation and metastasis in vitro and in vivo and suggested this combination as a powerful new therapeutic approach in EwS. However, imatinib as single agent therapy was not effective in EwS patients in two phase II studies conducted so far. Currently, metformin as addition to chemotherapy is tested for children with solid tumors in a phase I study (NCT01528046) as well as its potential use for maintenance therapy of children and adults with bone sarcoma (NCT04758000). Furthermore, Kilic et al*.* found GLUT1 expression downstream of HIF-1-a as well as the PI3K/Akt pathway that contributed to resistance of EwS cell line A-673 to chemotherapeutics such as doxorubicin, vincristine, and actinomycin D under hypoxia [7]. Of note, the A-673 cell line is a p53 deficient cell line, but the same hypoxia-induced drug resistance was also observed in the p53 wildtype rhabdomyosarcoma cell line A204 [2]. Further evidence for the involvement of the PI3K/Akt pathway in hypoxia-induced drug resistance in EwS is that the tyrosine kinase inhibitor imatinib reduced HIF-1-a levels in EwS cells and thereby reversed hypoxia-induced resistance to metformin [[11](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR39)]. Moreover, Magwere et al*.* demonstrated that hypoxia-induced drug resistance in EwS was heterogenous across different chemotherapeutics and cell lines, thus adding complexity to the topic [[12](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR37)]. Of note, glutathione (GSH) levels in response to hypoxia were also heterogenous across EwS cell lines, indicating that the GSH antioxidant system is probably not ideally suited for therapeutic targeting of hypoxia-induced drug resistance in EwS [5]. Finally, a recent study uncovered the NPY/Y5R-RhOA axis as potential mechanism of hypoxia-induced chemoresistance in EwS [[7](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR69)]. The authors demonstrated that Y5R inhibition successfully reduced hypoxia induced EwS disease recurrence in bones in vivo and thereby strongly underlined the rationale for targeting the hypoxic cell population within a EwS tumor [11].

**Hypoxia and/or HIF-1-a activity and EwS metabolism**

One of the hallmarks of cancer is the reprograming of the energy metabolism, to fuel its uncontrolled cell growth. In EwS, EWSR1::FLI1 mediated upregulation of enzymes involved in serine-glycine biosynthesis and glucose metabolism, as well as increased expression of glutamine transporters [5]. Furthermore, EWSR1::FLI1 inhibited the breakdown of tryptophan in the kynurenine pathway thus hindering aryl hydrocarbon receptor (AHR) signaling. HIF-1-a is known to play an important role as a regulator of cancer metabolism, mainly through shifting it from an oxidative to a glycolytic form. In cancer cells reciprocal upregulation exists between HIF-1-a and glycolysis [6]. Furthermore, HIF-1-a-mediated induction of glycolytic enzymes can arise independently from hypoxia, possibly explaining the Warburg effect [11]. Along these lines, aerobic glycolysis, characteristic of the Warburg effect, was found in EwS cell lines but not in chondrosarcoma or non-malignant cell lines [[7](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR71)]. Moreover, a direct link between HIF-1-a and aerobic glycolysis in EwS cells [3] as well as a direct link between hypoxia and GLUT-1 expression and glucose uptake [11] have been described. Additionally, when EwS cell lines were exposed to low glucose levels, a significant increase in HIF-1-a and HIF-2-a expression was found [[7](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR36)], illustrating again the potential for hypoxia-independent upregulation of HIF-1-a in EwS cells. Nevertheless, it is not yet elucidated if and how EWSR1::FLI1 and HIF-1-a act together to change EwS metabolism and how this may be potentially exploited for targeted therapies.

**Hypoxia and/or HIF-1-a activity and acidosis in EwS**

Increased glycolysis leads to intracellular and extracellular acidification and thus contributes to tumor acidosis, which was shown to be true in bone sarcomas [5]. Accordingly, HIF-1-a signaling seemed to be crucial in these events, regardless of whether HIF-1-a activation happened due to hypoxia or not [25]. Most interestingly, DiPompo et al*.* suggested that tumor acidosis could reciprocally influence HIF-1-a levels in bone sarcomas, for example via pH-dependent nucleolar sequestration of von Hippel-Lindau tumor suppressor (VHL) or nuclear factor kappa B (NF-kB) signaling, and for the later one evidence has already been found in osteosarcoma cells [7]. Avnet et al*.* found that EwS cells employed the V-ATPase proton pump to maintain pH homeostasis during tumor acidosis, suggesting V-ATPase as potential target for EwS treatment [4]. All in all, little is known about the role that hypoxia and HIF-1-a signaling play in EwS tumor acidosis, however the discussed studies suggest that further research in this field could open new therapeutic opportunities.

**Hypoxia and/or HIF-1-a activity and EwS vasculature**

Angiogenesis is the sprouting of new vessels from pre-existing ones and develops in response to tumor hypoxia. EwS cells replying to hypoxia promoted the release of angiogenic factors form the surrounding stroma and additionally expressed themselves VEGF, CXCR4, and fibroblast growth factors (FGFs) to bring on the angiogenic switch. One key regulator in this process was the zinc finger WT1 transcription factor (WT1): WT1 was upregulated in response to hypoxia, directly induced transcription of VEGF and thus assisted in angiogenic activities and tube formation of endothelial cells in EwS [4]. Vasculogenesis is the process in which bone marrow (BM) cells, endothelial cells, and pericytes/vascular smooth muscle cells (vSMC) organize to form the tumor vascular network [7]. A downregulation of delta like canonical Notch ligand 4 (DLL4) was correlated with reduced pericytes/vSMCs covering of the vessels, making them leak and increasing EwS hypoxia. Furthermore, repressor element 1-silencing transcription factor (REST), was identified to be a key regulator of EwS vessel proficiency. Intriguingly, low expression of this EWSR1::FLI1 target gene impaired EwS vessel morphology and increased tumor hypoxia. Lastly, the ability of tumor cells to form microvascular channels in hypoxic microenvironments is called ‘vascular mimicry’. HIF-1-a was highly expressed by EwS cells around blood lakes and could drive vascular mimicry in those tumor cells. Additionally, EwS cells surrounding blood lakes also expressed Y2R, implying involvement of Y2R and NPY in EwS vascular mimicry [9]. In summary, hypoxia and HIF-1-a have been found to promote vascular expansion in EwS throughout different mechanisms, highlighting their potential therapeutic value in EwS treatment.

**Hypoxia and/or HIF-1-a activity and EwS endochondral ossification**

EwS basically emerges in bones and hypoxia assumes a significant part during bone turn of events, explicitly the course of endochondral hardening (ECO) [20]. As a matter of fact, hypertrophic chondrocytes should defeat hypoxia to empower bone development, which they do by means of HIF-1-a flagging and enlistment of VEGF [7]. This presence of angiogenic factors in the microenvironment could eventually make a well-fitting soil for Ewing sarcomagenesis [26]. Besides, proof exists for crosstalk of EWSR1::FLI1 with different record variables of bone turn of events, for example, enlistment of SRY-box record factor 6 (SOX6) through EWSR1::FLI1 [22], the immediate restricting of EWSR1::FLI1 to RUNX family record factor 2 (RUNX2), and the circuitous impact of EWSR1::FLI1 on SRY-box record factor 9 (SOX9) guideline. On a comparable note, relationship of SOX6 and SOX9 articulation with hypoxia/HIF-1-a has been found with regards to bone development [26]. In light of these discoveries we recommend ECO-related hypoxia/HIF-1-a motioning as expected determinants in EwS pathogenesis, yet more exploration in this field is required. Of note, the bone specialty and the related hypoxic conditions as key variables impacting EwS pathophysiology have previously been examined. In like manner, hypoxia as a fundamental piece of the bone microenvironment pulled in EwS cells that had recently been exposed to hypoxia to metastasize explicitly deep down specialty in vivo however not to different compartments [11]. Besides, hindrance of the Y5R exactly decreased bone metastasis in vivo however not metastasis in different areas [7]. This underlines the significant job of the TME and shows intra-tumoral heterogeneity among EwS growth cells. Curiously, hypoxia was critical to produce EWSR1::FLI1-driven EwS models from human mesenchymal undifferentiated organisms got from an EwS patient. At long last, hypoxia or potentially HIFs assume a significant part for osteoclast excitement during bone resorption [4] and broad osteolytic bone obliteration has been known as a primary quality of EwS [27].

**Hypoxia and/or HIF-1-a activity and chromosomal instability in EwS**

CIN as continuing errors in chromosomal segregation during successive cell divisions [[4](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR131)] is a common phenomenon across cancer entities including EwS [[7](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR69)]. The resulting genomic instability promotes tumor cell adaptation to harsh environmental conditions and probably confers aggressiveness to EwS tumors. Most interestingly, hypoxia causes CIN and aneuploidy in EwS cells via the NPY/Y5R-RhoA-axis. This might ultimately increase EwS disease recurrence and metastatic potential [[7](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR69)]. Of note, EwS cells that were exposed to hypoxia keep their tendency for mitotic segregation errors and CIN even upon reoxygenation, indicating that EwS cells keep a cellular memory of having been exposed to hypoxia [[28](https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-023-01750-w#ref-CR69)].

**Conclusion**

This survey sums up arising proof that hypoxia and HIF flagging are associated with EwS pathophysiology in more ways than one, e.g., in movement and metastasis, digestion, and development of vasculature, featuring the significance of concentrating on them. In light of past reports, we presented the idea of review hypoxia and HIFs freely from one another while taking a gander at sub-atomic cooperations of HIF-1-an and EWSR1::FLI1, yet this speculation should be additionally approved. Furthermore, have displayed in our EwS patient partner that statement of HIF-1-an and downstream targets is related with more terrible visualization, basic the clinical pertinence of hypoxia and HIFs in EwS.

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