Chapter 10

The Hypoxia-Inducible Factor-1α in Angiogenesis and Cancer: Insights from the *Drosophila* Model

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Abstract

The hypoxia-inducible factor-1α (HIF-1α) is an evolutionarily conserved transcription factor with prominent roles in the hypoxic response, cell survival, angiogenesis and cancer. HIF-1α functions as a sensor of molecular oxygen: in the presence of oxygen, it is degraded by the proteasome, whereas in reduced oxygen tensions, it heterodimerizes with the constitutively expressed HIF-1b subunit forming the functional HIF1 transcription factor, which enters the nucleus to control expression of hypoxia-inducible genes. Since HIF-1α has been found upregulated in several cancers, it has attracted a lot of clinical interest, because it represents an interesting candidate for pharmacological chemotherapy interventions. In this chapter, we discuss our current knowledge on the HIF1 transcription factors and their major roles in development, physiology, angiogenesis and cancer using examples of recent studies in the model organism *Drosophila melanogaster*. Given the striking functional conservation between the mammalian and fruit fly HIF-1α, we expect that future studies in the *Drosophila* model will not only expand our knowledge on the basic HIF1 biology, but they will also pinpoint conserved molecular regulators of HIF1 that might lead to the discovery of novel cancer therapeutics.

Keywords: hypoxia, tumorigenesis, Warburg effect, metabolism, tracheogenesis, inflammation, *Drosophila*

1. HIF-1α in mammalian angiogenesis, inflammation and cancer

1.1. Oxygen is required for survival of all animals

Oxygen (O_2) is the main ingredient of the atmospheric air and is required for the survival of all living organisms. It is also present in the seas and oceans, and it is necessary for survival of all aquatic living organisms. Oxygen accumulated on Earth's atmosphere about 2.5 billion

years ago [1]. However, it was discovered only 245 years ago, by the chemist Carl Wilhelm Scheele [2]. Its main role in the survival of animals derives from its utilization during cellular respiration. Specifically, oxygen is involved in oxidative phosphorylation, the process that transfers the chemical energy stored in carbon bonds to the phosphate bonds of Adenosine Tri-Phosphate (ATP), which is the main energy carrier in all cell types of living organisms [3]. In addition, oxygen is the main component of ATP production, because it is the final electron acceptor of the respiratory chain. Oxygen-electron reaction leads to the release of reactive oxygen species (ROS), which, when accumulated, results in oxidative stress and eventually cell death [4, 5]. Oxygen is necessary as an energy substrate, and the danger of oxidative damage needs to be kept at equilibrium. Therefore, oxygen homeostasis is critical for all cellular processes, and its intermittent supply results in many pathophysiological conditions, such as myocardial ischemia, sepsis, pulmonary hypertension, chronic obstructive pulmonary disease and cancer, which correspond to frequent causes of mortality in the Western world [6].

The ambient oxygen concentration is 21%, and the cells of the majority of healthy tissues are exposed to less oxygen, which varies between 2 and 16% [6, 7]. Normoxia is defined as normal oxygen levels, whereas hypoxia is a situation where the organism is underprivileged of sufficient oxygen supply. Hypoxia can be continuous or intermittent [4]. In anoxia, oxygen levels are strictly or totally insufficient, and in hyperoxia, oxygen is superfluous in the tissues and organs of the body. Low oxygen levels are mostly observed in acute inflammatory conditions and also within solid tumors [7]. In contrast, hyperoxia might be the result of immoderate oxygen delivery to the organism due to unlimited angiogenesis [8].

Transport of oxygen throughout the bodies of animals is achieved via different mechanisms that depend on the living environment and the size of each organism. For example, in small animals, such as the nematode *Caenorhabditis elegans*, atmospheric oxygen enters the body via diffusion. Insects, such as *Drosophila melanogaster*, use an elaborate network of tubules, and the tracheal system transports oxygen from the outside environment via the spiracular openings to all the cells of the body. Specialized cells of the insect tracheal system, the terminal cells, are involved in actual gas exchange and come in close contact with different cells in the body that need oxygen. The respiration process and the allocation of oxygen to the trillions of cells in the bodies of organisms, such as vertebrates, seem to be more intricate because of their large body size. The delivery of oxygen to each part of the human/vertebrate body is achieved through the lungs, the diaphragm, the erythrocytes, the heart and the vasculature [3, 9].

1.2. The transcription factor HIF1 is key in oxygen sensing

The hypoxia-inducible factor-1α, HIF-1α, was characterized in 1990 as a transcription factor with a key role in oxygen sensing [10]. The discovery of HIF-1α opened new research avenues focusing on oxygen sensing and oxygen poverty [10]. Despite the discovery of two additional HIFs (the HIF-2α & the HIF-3α) and the variable response of all HIFs to hypoxia, HIF-1α remains the molecule with the major role in oxygen sensing [11, 12].

The importance of HIF-1α and oxygen sensing for living organisms is underscored by the evolutionary conservation of this transcription factor in animals. The genomes of different species ranging from corals to insects to mammals (i.e., *Acropora millepora, Nematostella vectensis, Caenorhabditis elegans, Palaemonetes pugio, Drosophila melanogaster*, *Anopheles gambiae, Apis mellifera*,

Nasonia vitripennis, *Eurosta solidaginis*, *Tribolium castaneum*, *Mus musculus*) encode homologs of the HIF-1α [13]. HIF-1α is present in all metazoans [14] and is characterized as a master regulator of hypoxia-inducible genes in mammals [15]. HIF1 is a heterodimer of two subunits: one labile oxygen-sensitive subunit, the HIF-1α and one stable constitutively expressed subunit, the HIF-1β [3, 16–18]. The HIF subunits form a subfamily of the basic-Helix-Loop-Helix-Per/ARNT/Sim (bHLH-PAS) superfamily of transcription factors. The bHLH proteins comprise a superfamily of eukaryotic transcription factors that can dimerize via their HLH domain, and the bHLH-PAS proteins are only a small group of this superfamily [18, 19]. The HIF1 heterodimer forms a bHLH transcription factor that recognizes and binds the hypoxia-response elements (HREs) on DNA. HREs are present in the promoters of HIF target genes, which are involved in intracellular homeostatic processes, such as energy metabolism, angiogenesis, erythropoiesis and apoptosis [15, 17]. HIF-1α is the main sensor of low oxygen concentrations [20] and is stabilized upon hypoxia and also in response to divalent cations and iron chelators [21].

The human HIF-1α protein is composed of eight regulatory domains: the bHLH DNA binding and dimerization domain, the PAS dimerization domain, the amino-terminal and carboxy-terminal nuclear localization signals (NLS-N and NLS-C), the proline-serine-threonine-rich protein stabilization domain (PSTD), the amino-terminal and the carboxy-terminal transactivation domains (TAD-N and TAD-C), and the transcriptional inhibitory domain (ID). The HIF-1α peptide consists of 826 amino acids, whereas the HIF-1β peptide is smaller with 774 amino acids, because of alternative splicing in a region that encodes 15 residues [9]. Thus, the two HIF1 subunits have highly conserved amino acid sequences for the majority of the regulatory domains described above [22, 23]. Apart from HIF1 (HIF-1α and HIF-1β), there are also another two HIFs, the HIF-2α and the HIF-3α, also known as ARNT2 and ARNT3. Their expression is more restricted in human and mouse tissues compared with the HIF-1α and the HIF-1β subunits [24–26].

The expression of functional HIF-1α is controlled at multiple levels, such as transcription, nuclear transport, protein stability, and transactivation. Most of the studies have focused on the stabilization of HIF-1α protein at *in vivo* and *in vitro* changes of oxygen levels [9]. Under sufficient oxygen concentrations (normoxia, 21% O_2), the von Hippel-Lindau (VHL) E3 ubiquitin ligase recognizes and binds to the hydroxylated HIF-1α subunit. VHL binds to HIF-1α only when HIF-1α is hydroxylated by the prolyl-4-hydroxylase (PHD). PHD operates as a direct sensor of oxygen, because it uses O_2 as a substrate and attaches –OH groups to particular proline residues (Pro402 and Pro564 of human HIF-1α) [27]. Binding of VHL recruits a ubiquitin ligase complex composed of Elongin C, Elongin B, Cullin 2 (Cul2), and a Ring box protein (Rbx1) and attracts a ubiquitin-conjugating enzyme that attaches a polyubiquitin chain to HIF-1α to target it for proteasomal degradation [17]. The Pro564 residue of HIF-1α has a higher affinity for PHDs compared to the Pro402 residue [28]. However, the hydroxylation of the one can affect the hydroxylation of the other [28]. Other factors that play critical roles in the regulation of the pathway is the availability of O_2 and Fe(II), and additionally, whether VHL protein can function properly or whether it has a mutation that makes it dysfunctional [28]. Furthermore, the control of the process is related to the cell type and the developmental phase of the tissue or organ. This regulation can be affected by post-translational events [28].

In hypoxia (1–2% O_2), the PHD enzyme cannot hydroxylate HIF-1α because oxygen is lacking, VHL cannot bind to the HIF-1α subunit, and therefore, HIF-1α is not degraded by the proteasome [20]. Stabilized HIF-1α is quickly transported to the nucleus to induce the transcription

of target genes [20, 29]. In the nucleus, the HIF-1α/HIF-1β heterodimer binds the p300 coactivator [30]. HIF1 and p300 form a complex that binds the double-stranded DNA and promotes transcription. HIF-1α has a plethora of target genes, which encode proteins involved in critical biological processes, such as erythropoiesis, vascular remodeling, metabolism, cell proliferation, cell viability and angiogenesis [9, 10] (**Table 1**).

1.3. HIF-1α controls tumor angiogenesis

Angiogenesis is the process of forming new blood vessels from pre-existing ones, which differentiates into a vascular network [53, 54]. Blood vessels supply the body with oxygen, nutrients, and immune surveillance. The extensive growth of veins and their non-physiological remodeling result in multiple illnesses, such as cancer and ischemic and inflammatory diseases (e.g., arthritis, atherosclerosis, and diabetes) [54–58]. The veins are used as pathways for the migration of cancer cells [54]. Angiogenesis may be adversely affected by infection with pathogenic bacteria. Additionally, angiogenesis is a feature of cancer, as tumor cells induce the process in order to grow and become metastatic [59].

Previous studies have shown a correlation between tumor growth and angiogenesis and have established molecular links between the signaling pathways induced upon infection, gene regulation, and cancer [60–63]. According to the angiogenesis dogma, a tumor cannot grow more than a few millimeters in diameter, if it does not come in contact with the blood vessels by which it receives enough oxygen [54, 61]. Furthermore, due to the irregular shape and organization of the tumor vasculature, some cells are more than 100 mm away from the blood vessels and they also become hypoxic. The oxygen within the tumor is not static but fluctuates spatially and temporally [64]. Angiogenesis is regulated by molecules that act as "activators" (pro-angiogenic factors) or "inhibitors" (anti-angiogenic factors) [65]. Several studies have shown that the angiogenic activators play an important role in the growth and spread of tumors [66]. Key activators of angiogenesis belong to the family of VEGFs, and their receptors were found expressed in about half of human cancers investigated so far [66].

Importantly, HIF-1α has been shown to control the expression of proangiogenesis regulators, such as VEGF and other growth factors and often activation of their respective pathways feedback to enhance HIF-1α activity [9]. For example, the epidermal growth factors (EGFs) act as angiogenesis activators. The binding of EGF to the epidermal growth factor receptor (EGFR) activates the MAP kinase cascade and also induces the PI3K (phosphatidylinositol 3-kinase)—AKT/PKB (Protein Kinase B) pathway. The PI3K enzyme catalyzes the transfer of a phosphate group, which converts PI phospholipid (phosphatidylinositol) into PI-3P phospholipid (Phosphatidylinositol 3-phosphate). This conversion results in the full activation of the serine/threonine kinase PDK-1 (Phosphoinositide-dependent kinase), which phosphorylates and activates another serine/threonine kinase, known as AKT. PTEN (phosphatase and TENsing homolog), which functions as a kinase with tumor suppressor activity, is a negative regulator of PI3K, which mediates cell proliferation [67]. The protein kinase p70S6 is a target of mTOR. Through phosphorylation, it induces the translation of mRNAs, which encompass a 5′ end rich in pyrimidines. Such sites are found in the HIF-1α mRNA [68].

1.4. Tumor hypoxia, HIF-1α, and the Warburg effect

The hypoxic regions of a tumor are resistant to chemotherapy, exhibit modified metabolism, and often acquire metastatic and invasive properties [69–71]. Chronic cell proliferation, which

appears to correlate with tumor incidence, does not only involve cellular dysfunction but also energy metabolism adjustments through which the organism acquires enough energy by producing ATP, which is used by cancer cells for cell division and growth. In 1924, the Nobelist Otto Warburg first described the preference of cancer cells to convert glucose into lactic acid

Gene name	Gene symbol	Gene function	NCBI Gene ID	Refs
Angiopoietin 1	*ANGPT1 ANGPT2*	Angiogenesis	284	[31]
Angiopoietin 2			285	
BCL2 interacting protein 3	*BNIP3*	Apoptosis	664	[32, 33]
BCL2 interacting protein 3-like	*BNIP3L/NIX*	Apoptosis	665	[33]
Endothelin-1	*ET1*	Angiogenesis	1906	[34]
Enolase 1	*ENO1*	Energy metabolism	2023	[35]
Erythropoietin	*EPO*	Proliferation, survival	2056	[36]
Glyceraldehyde-3-phosphate dehydrogenase	*GAPDH*	Energy metabolism	2597	[37]
Glucose transporter-1	*GLUT1*	Energy metabolism	6513	[38]
Hexokinase 1	*HEX1*	Energy metabolism	3098	[39]
Hexokinase 2	*HEX2*		3099	
Prolyl 4-hydroxylase subunit alpha 1	*P4HA*	Energy metabolism	5033	[40]
Insulin growth factor 2	*IGF2*	Growth and survival	3481	[41]
Insulin growth factor binding protein 1, 2 and 3	*IGFBP-1*	Growth and survival	3484	[41, 42]
Lactate dehydrogenase A	*LDHA*	Energy metabolism	3939	[35]
MAX interactor 1	*MXI1*	Apoptosis, c-Myc activity	4601	[43, 44]
Nitric oxide synthase 2	*NOS2*	Angiogenesis	4843	[45]
Pyruvate kinase M	*PKM*	Energy metabolism	5315	[35]
Pyruvate dehydrogenase kinase 1	*PDK1*	Energy metabolism	5163	[46, 47]
Transforming growth factor beta 3	*TGF-b3*	Invasion, metastasis, growth, survival	7043	[48]
Tumor protein p53	*p53*	Apoptosis	7157	[49]
Twist	*TWIST*	Metastasis	7291	[50]
Vascular endothelial growth factor A	*VEGFA*	Angiogenesis, growth, survival	7422	[51]
Vascular endothelial growth factor receptor 1	*VEGFR-1/ FLT-1*	Angiogenesis, growth, survival	2321	[52]

Although several genomic studies have identified a plethora of potential HIF1 targets, here we focus only on direct targets of HIF-1α with characterized HREs

Table 1. A list of HIF-1α targets with key functions in a variety of physiological cellular processes, such as angiogenesis, survival, and energy metabolism.

even in the presence of oxygen [72]. By measuring lactic acid production and oxygen consumption in thin sections from healthy and tumorous rat livers, he concluded that normal liver cells inhibit the production of lactic acid in the presence of oxygen, whereas cancer cells produced lactic acid irrespective of the availability of oxygen [73, 74]. In aerobic conditions, normal cells convert glucose to pyruvic acid via glycolysis in the cytoplasm, and then, pyruvic acid is used in the mitochondria to produce acetyl Coenzyme A (CoA) and carbon dioxide (CO_2) during oxidative phosphorylation. In anaerobic conditions, normal cells favor glycolysis, and pyruvic acid is used in the cytoplasm to produce lactic acid. Instead, according to Warburg, cancer cells change their metabolism, and even in the presence of oxygen, glucose enters glycolysis and produces lactic acid. Cancer cells use 10 times more glucose than the amount of the cellular breathing process can use, while the amount of lactic acid produced is two times greater than that produced by healthy cells [73]. This phenomenon is known as the "Warburg effect" or "aerobic glycolysis" [70, 75–77]. At first sight, this phenomenon seems paradoxical, since aerobic glycolysis produces significantly less energy (4 mol ATP/mol glucose) compared to oxidative phosphorylation (36 mol ATP/mol glucose). Nevertheless, cancer cells exhibit an increased expression of glucose transporters, such as GLUT1, which correlates with enhanced glucose uptake [78–80]. The feeding of cancer cells with glucose is often associated with oncogene activation and loss-of-function of tumor suppressor genes [78, 79, 81]. The *myc* oncogene is an important regulator of cancer metabolism, since among its many targets, are those of GLUTs as well as genes encoding pyruvate dehydrogenase kinase 1 (PDK1) and lactate dehydrogenase A (LDHA) that promote the Warburg effect by increasing the flow of glucose through glycolysis, while inhibiting the entry of pyruvic acid into the Krebs cycle [82–84]. Furthermore, both the oncoprotein Ras and hypoxia can independently increase the levels of the HIF-1α and HIF-2α transcription factors, which in turn positively regulate glycolysis [85–87]. In addition, loss-of-function mutations in tumor suppressor genes, such as *vhl* [88] and *p53* [89, 90], lead to elevated levels of HIF-1α and VEGF. Gain-of-function mutations in oncogenes, such as the *v-src* [91], activation of EGF, and insulin growth factor I (IGF-I) receptors, also induce HIF-1α [41, 92].

A series of major discoveries remained as milestones in the field of cancer biology followed Warburg's observations. These include the purification and cloning of the HIF-1 in 1995 [36], the effects of HIF-1 in cancer progression in mice [93], the description of VHL [94], the identification of the PHD enzymes, and the establishment of the HIF-α subunit prolyl hydroxylation [69]. The area of hypoxia remains an attractive subject for intensive research, although over a century has passed, since it was first taken into account. With the discovery of HIFs, an extremely attractive field of research emerged and novel proteins came into play, such as the glucose regulated proteins (GRPs), oxygen regulated proteins (ORPs), PDGF, interleukin-1α (IL-1α), endothelin-1, VEGF and erythropoietin (EPO) [95–100]. The characterization of HIF-1 led to the discovery of upstream activators and downstream signals as potential new therapeutic targets. Such targets include the VEGF, fibroblast growth factor (FGF), TGFα, the PI3K/AKT/mTOR and RAS signaling pathways [101]. In addition, reduced oxygen tensions can repress mTOR in the cells similar to the effects of rapamycin. mTOR in hypoxic environments acts as an oxygen sensor and leads to reduced protein translation [102].

The PI3K/AKT pathway inhibits programmed cell death and alters cell proliferation [103]. Loss of PTEN, which is a negative regulator of the pathway, can lead to increased angiogenesis

in the case of prostate cancer. This has been associated with the induction of HIF-1α that guides elevated VEGF expression [103, 104]. In colon tumors, transfection of cells with a HIF-1α expression vector resulted in elevated VEGF mRNA levels and increased angiogenesis [90]. The EGF/PI3K/AKT/TOR pathway promotes VEGF and the transcriptional activity of HIF-1α protein in prostate cancer [89]. Chemical inhibitors of PI3K and TOR, the LY294002 and rapamycin, respectively, inhibited growth factor-induced and mitogen-induced secretion of VEGF. This connected the PI3K/PTEN/AKT/TOR pathway with HIF1 and the process of angiogenesis [105]. In the absence of HIF-1α, the development of a tumor is dramatically reduced although not completely stalled [106]. Moreover, HIF-1α is overexpressed in different cancer types, such as colon, breast and lung carcinomas. HIF-1α is also overexpressed in preneoplastic, premalignant adenomas and other intraepithelial neoplasia and also in malignant and metastatic tumors [89]. Therefore, discovery of chemicals that could potentially control the HIF-1α pathway is of major clinical importance.

1.5. HIF-1α and inflammation

Another important aspect of HIF in tissue maintenance is its role in regulating inflammation and innate immunity. HIFs appear to have different functions in different immune cell types. For example, HIF-1α mediates bacterial killing via regulation of pro-inflammatory gene expression in macrophages [107]. On the other hand, in the case of neutrophils, HIF-1α promotes cell survival upon hypoxia and promotes extensive angiogenesis which is regulated by β2-integrin expression. Furthermore, there is a link between the effect of HIFs in immune cells, inflammation, and tumorigenesis [107]. It is known that HIF-1α is regulated by the availability of oxygen. Interestingly, not only hypoxia but also bacterial products, such as cytokines and growth factors, induce HIF-1α. Inflammatory cytokines, such as tumor necrosis factor a (TNF-α) and interleukin-1β (IL-1β), induce HIF-1α transcription. TNF-α induces HIF transcription and the Nuclear Factor-kappa B (NF-κB) pathway is needed for stabilization of the protein [108, 109]. In the case of IL-1β, the stability of HIF is promoted by the activation of NF-kB activity and the inhibition of VHL function [110]. There is an important crosstalk between NF-kB and HIF upon inflammation and cancer [111]. NF-kB is activated in inflammatory conditions, including cancer, and the activation of the pathway is a characteristic of inflammatory disease [59]. Its role in malignant situations is controversial such that it can act both as a tumor promoter and as a tumor suppressor [112]. Its activated form is implicated in excessive cell proliferation, metastasis, inhibition of apoptosis and angiogenesis [112]. Importantly, cells expressing wild-type p53 undergo apoptosis in hypoxic conditions, in contrast to the mutant p53 cells that are resistant to apoptosis. These results reveal that HIF-1α can promote cell proliferation and thus tumorigenesis by inhibiting apoptosis [113]. The chemokine interleukin-8 (IL-8) [114] and the VEGF [115] NF-kB target genes promote angiogenesis. Importantly, these are also targets of HIF-1α [116, 117], revealing that there is a crosstalk between NF-kB and HIF-1α. This crosstalk is bi-directional, because although NF-kB promotes the activation of HIF, HIF restricts the transcriptional activity of NF-kB [117]. Inflammation promotes NF-kB activity, which leads to tumorigenesis [118]. Not only NF-kB, but also other transcription factors (e.g. STAT3) can induce HIF [119]. PHDs antagonize NF-kB in different tumor cells [120–122]. In colorectal cancer, NF-kB promotes tumorigenesis. Different signaling pathways drive its oncogenic role.

These pathways regulate the production of ROS, the activation of pro-inflammatory cytokines, the uncontrolled cell proliferation, migration, metastasis and angiogenesis [123]. The absence of NF-kB has a negative effect on tumor progression in mouse models of colorectal cancer [124]. ROS affect the hydroxylation of HIF-1α and, thus, modify its activity [125, 126]. Specific defects, not necessarily mitochondrial defects, that restrict the consumption of oxygen, result in enhanced prolyl-hydroxylation accompanied with reduced HIF levels [125, 127].

The induction of HIF by such inflammatory cytokines indicates that HIF has a crucial role in inflammatory responses. Apart from inflammatory cytokines, various signaling pathways seem to also have important roles in the stimulation of HIF. Such pathways include PHDs [128], NF-κB [129–131], MAPKs [130], and ROS [129]. In addition, ROS are released by the mitochondria as a cause of low oxygen tensions, and they can control transcriptional and posttranslational events [132]. Another important crosstalk in colorectal cancer is that between HIF-1α, β-catenin and APC: when repressed under insufficient oxygen levels, APC can lead to activation of the Wnt/β catening signaling and increased proliferation that drives tumorigenesis [133]. Interestingly, NF-kB is regulated by Wnt/β-catenin [134].

2. The HIF-1α pathway in *Drosophila melanogaster*

2.1. The HIF-1α pathway is conserved in *Drosophila*

The *Drosophila melanogaster* genome encodes homologs of the core proteins involved in the HIF-1α pathway. For example, there are two HIF-1α homologs in fruit flies, one is encoded by the gene *similar* (*sima)* and its paralogue is known as *trachealess (trh)* [20, 27, 135]. Sima responds to changing oxygen levels, whereas Trh acts as a patterning gene during *Drosophila* development. The HIF-1β homolog in flies is the product of the gene *tango* (*tgo*) [136]. Both *sima* and *tgo* are transcribed during larval stages [136]. Although Tgo is expressed uniformly throughout development [137], Sima accumulates in the majority of tissues only in hypoxic conditions [138]. Tgo and Sima heterodimerize to control transcription in environments with decreased oxygen levels [139, 140].

Sima has a molecular weight of 180 kDa, is larger compared to the mammalian HIF-1α, and bears 45% similarity in the PAS domain and 63% in the bHLH domain with its human homolog (**Figure 1**) [141, 142]. The single prolyl-4-hydroxylase (PHD) enzyme homolog in *Drosophila* is encoded by the gene *fatiga* (*fga*) and acts as an oxygen sensor. Fga uses O_2 as a substrate and hydroxylates a single Pro residue in Sima (Pro850) [143]. Another factor that plays crucial role in the process is the availability of Fe (II) [28]. In normoxia, where O_2 is abundant, Sima is hydroxylated and targeted to the proteasome for degradation via association with the von Hippel-Lindau ubiquitin ligase, which in *Drosophila* is encoded by the dVhl gene [20]. In low oxygen tensions (environmental hypoxia and tumors), the HIF-1α/Sima transcription factor is not degraded, because it cannot be hydroxylated by Fga due to the lack of oxygen. Consequently, HIF-1α/Sima binds to the constitutively expressed HIF-1β/Tgo forming the HIF1 heterodimer, translocates to the nucleus and binds to hypoxia-response elements (HREs) to control transcription of target genes [20, 29] (**Table 2**).

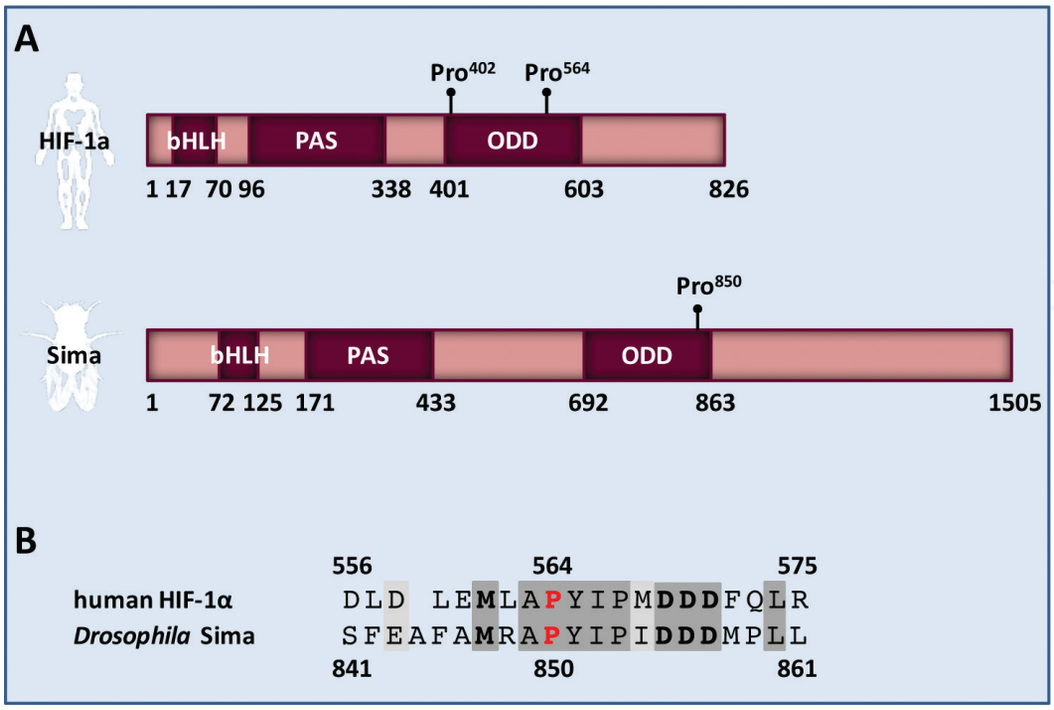

Figure 1. The human HIF-1α and *Drosophila* Sima are homologous proteins. (A) The human HIF-1α protein has a length of 826 amino acids, whereas its *Drosophila* homolog Sima is much larger and consists of 1505 amino acids. The shared functional domains of the two proteins are indicated with darker color: the bHLH DNA binding and dimerization domain, the PAS dimerization domain and the oxygen-dependent degradation domain (ODD). Two Pro residues (Pro^{402} and Pro^{564}) are substrates of the propyl-4-hydroxylase PHD in the human HIF-1α, whereas in *Drosophila*, the propyl-hydroxylase Fga targets a single Sima Pro residue (Pro^{850}). (B) Amino acid alignment of the human HIF-1α (amino acids 556–575) and the *Drosophila* Sima (amino acids 841–861) highlighting in red the hydroxylation targets Pro^{564} and Pro^{850}, respectively. Dark gray and light gray boxes indicate identical residues and conservative amino acid substitutions in the two proteins, respectively.

Gene name	Gene symbol	Human homolog	Gene function	NCBI Gene ID	Refs
branchless	*bnl*	*FGF10*	Tracheal development, cell migration	42356	[144]
breathless	*btl*	*FGFR*	Tracheal development, cell migration	39564	[144]
Ecdysone-inducible gene L3	*ImpL3*	*LDHA*	Energy metabolism	45880	[145]
fatiga B	*fgaB*	*HPH3*	Energy metabolism	40633	[146]
heat shock factor	*hsf*	*HSF1*	Heat response, defense response	37068	[147]

Table 2. A list of Sima targets identified by genetics and direct binding assays.

2.2. HIF-1α/Sima controls remodeling of the tracheal gas-transporting tubes

In *Drosophila melanogaster*, an extensive network of interconnected tubes, the tracheal system, transfers oxygen throughout the body. The *Drosophila* trachea is therefore functionally analogous to the mammalian respiratory system [148]. It is responsible for the oxygenation of the

flight muscles, the brain and all internal organs, such as the intestine. The tracheal system of the fly is a good model for studying tracheal cell migration and the mechanisms that direct the movement of cells in different directions [149]. Systematic studies of the molecular markers labeling the trachea, mutational studies on specific genes and cellular imaging have identified the basic steps in the development of the tracheal system. Embryonic tracheal morphogenesis is initiated at stage 10 of embryogenesis and proceeds through four sequential steps: the formation of the tracheal placode and the sprouting of the primary, secondary and tertiary branches [148]. Tracheal cell specification and primary and secondary branch formation are genetically controlled stereotypical processes, whereas terminal branching is environmentally controlled and adjusted according to the needs of the tissue for oxygen [148]. After embryogenesis and during larval life, the fly trachea grows in size to accommodate the increased oxygen needs of the larva. At the same time, specialized airway progenitors, the tracheoblasts, get activated to proliferate and differentiate to remodel the pupal and adult tracheal system during metamorphosis [150–154]. The FGF/FGFR pathway controls all aspects of tracheal morphogenesis. In flies, the FGF homolog encoded by the gene *branchless* (*bnl*) and the FGFR homolog encoded by the gene *breathless* (*btl*) are repeatedly utilized for tracheal development and remodeling. Bnl/FGF acts as a chemoattractant that can direct tracheal sprouting in cells expressing the Btl/FGFR [155–157].

Remarkably, *Drosophila* adults and larvae present a different mode/pattern of response during hypoxia. In adult *Drosophila*, this response includes the opening of the spiracles, the aeration of the body and the transposition of the fluids from the tracheoles [158, 159]. It has been shown that the majority of insects can survive in complete anoxia for quite a long period in contrast to mammals. The survival of adult *Drosophila melanogaster* under anoxia without any tissue damage and reaches a period of about 4 hours [160]. This time interval depends on the developmental stage of the animal. For example, larvae illustrate escape mobility for 20 minutes in these conditions [161], whereas adult *Drosophila* remains stationary within a period of only 60 seconds, and this is because of the effacement of the electrical responses of the insect muscles [160]. The retention of flies in anoxia for 12 hours leads to death [162]. Furthermore, the survival of the flies in anoxia depends on the suppression of ATP synthesis, with simultaneous reduction of the harmful reverberations of low energy availability [161]. Interestingly, although flies challenged for 6 hours in 0.5% O_2 stop responding and remain motionless, following their reoxygenation, they behave physiologically without any defects [163]. Moreover, the behavior of *Drosophila* under low oxygen conditions depends on the degree of hypoxia and whether it is constant or intermittent [164]. Different gene families have been found regulated in different types of hypoxia. For example, in flies that experienced intermittent hypoxia, a smaller proportion of altered genes has been observed, in comparison with the flies that experienced constant hypoxia. The same flies revealed decreased metabolic rates and loss of spiracular control [165, 166]. In *Drosophila*, the adaptation to hypoxia involves mechanisms that increase oxygen delivery, such as the expansion of the spiracular openings, as noted above, that are able to propel oxygen to the whole organism [158]. The expression of HIF1 increases the diameter of tracheal tubules and induces the expansion of cells that directly contact target tissues, the tracheoles [167].

Interestingly, the embryonic and larval fly trachea encompasses specialized cells, which extend cytoplasmic processes to carry oxygen to the tissue. These cells, known as tracheal terminal cells, are very similar to the tip cells of the mammalian blood vessels; they are plastics and they

respond to hypoxia by extending cytoplasmic tubular processes, the terminal branches, toward the hypoxic tissue [148]. The sprouting and growth of the terminal branches are carefully adjusted according to the needs of tissue in oxygen, just as in the case of sprouting angiogenesis in mammals. Hypoxia induces terminal branching, whereas hyperoxia (increased oxygen supply) suppresses the formation of terminal branches [148, 168]. Hypoxia induces the expression of *bnl*/*FGF*, which acts as a chemoattractant that can direct the newly formed branches of every cell that expresses the FGFR/Btl [148]. The formation of new branches depends on the HIF-1α homolog Sima and the HIF-propyl hydroxylase Fga, which acts as an oxygen sensor [144]. In hypoxia, HIF-1α/Sima accumulation in tracheal cells induces the expression of *btl*/*FGFR* and thus causes further sprouting of new branches, whereas in nontracheal cells, Sima contributes to the induction of *bnl*/*FGF* [144]. Therefore, the hypoxia-induced trachea-specific *btl*/*FGFR* expression, probably, enhances their sensitivity in the presence of high levels of Bnl [144]. In nontracheal target tissues, such as the larval muscle, the Archipelago (Ago) F-box/WD-repeat protein substrate specificity factor for a Skp/Cullin/F-box (SCF)-type polyubiquitin ligase has been shown to antagonize HIF-1α/Sima-dependent *bnl*/*FGF* expression. Ago physically associates with HIF-1α/Sima, reduces its levels, and inhibits the hypoxic response [29, 169]. Thus, terminal cell remodeling in *Drosophila* is controlled by the evolutionarily conserved HIF-1α/Sima pathway, similar to tip cell remodeling of mammalian blood vessels [136, 170].

2.3. HIF-1α/Sima and growth control in *Drosophila*

Insects have a mechanism of body size plasticity. Oxygen sensing has a major role in this mechanism. Hypoxia causes a reduction of body size in the fruit fly *Drosophila* and the moth *Manduca* [171]. The primary regulators of this phenotype are the HIF1 and nitric oxide synthase (NOS) signaling pathways, which are activated in hypoxia. In normoxia, NO inhibits HIF-1α at the level of protein hydroxylation that targets it to the proteasome for degradation [171].

Over the last decade, many scientists tried to address the role of HIF in cell growth and cell size control. Overexpression of *HIF-1α*/*sima* in the fat body resulted in smaller cells compared to the control wild type cells, indicating that Sima operates as a cell-autonomous negative growth regulator [140]. In addition, *fga* mutant pupae revealed reduced rate of growth and smaller size compared to wild type [140]. Cells with *fga* loss-of-function in the larval fat body were found smaller compared to the wild type cells in the same tissue [172]. In contrast, overexpression of the same gene in the wing imaginal discs led to the growth of the cells [140]. HIF uses at least two mechanisms to control the cellular growth in *Drosophila*. It can block protein synthesis, by targeting the insulin-like peptide (ILP)-TOR-S6 K pathway, and as a consequence, there is a reduction of cellular growth of the whole animal. This has been so far seen in the fat tissue, the eye and the gut of *Drosophila* [173]. In addition, *Drosophila* mutants overexpressing or lacking the gene *fga* revealed that HIF controls the function of the cyclin-dependent protein kinase 4 (Cdk4), which is responsible for the activation of cellular growth [172]. Moreover, HIF is also implicated in the expression of *scylla* and *charybdis* genes that downregulate the S6 K-dependent activation of protein synthesis, and this results in the reduction of cellular and body growth [173].

It is firmly established that insulin growth factors and components of the insulin pathway upregulate the HIF-1α protein, thus promoting the expression of hypoxia-sensitive genes

[174]. The PI3K/Akt/TOR signaling pathway is directed by insulin to induce the transcription of *HIF-1α*/*Sima* both in S2 cell lines and in *Drosophila* embryos [175]. Thus, the transcriptional activation of HIF-1α/Sima is guided by the insulin receptor (InR)-regulated PI3K-AKT and TOR signaling pathways [175]. RNAi silencing experiments showed that the mRNA levels of *HIF-1α*/*sima*, upon induction with insulin, depended on those two pathways. Experiments in fly embryos, where components of these pathways were overactivated, also revealed the upregulation of *sima* mRNA levels [175]. As noted above, the overexpression of *sima* in different tissues of the fly resulted in the reduction of cell size in these specific tissues [140]. But this is not consistent with our knowledge about the role of PI3K-AKT and TOR pathways in correlation with Sima and cell growth. Particularly, it is known that the activation of these pathways induces growth in different levels, and in parallel with this, it also induces the transcription of genes that are targeted by Sima. However, Sima is a negative regulator of growth. It seems like there is a negative feedback loop, in which the two pathways upregulate growth and Sima simultaneously, and then, Sima downregulates the two pathways, and this consequently results in growth limitation [176]. Interestingly, Sima induces *scylla* in hypoxic conditions, which in turn feeds back on the TSC1 complex to inhibit the TOR pathway and growth. Thus, s*cylla* seems to be part of the negative feedback loop that coordinates InR-mediated growth with HIF-1α/Sima induction during hypoxia [173].

2.4. Other functions of HIF-1α/Sima in *Drosophila*

2.4.1. Epithelial cell migration

A 2010 study dealt with the role of Sima in the rate of cell migration and invasion of the ovarian border cells in *Drosophila*. In more detail, the researchers studied the role of hypoxic response and HIF-1α/Sima during invasion and metastasis. It was shown that the HIF pathway controlled the rate or invasion in the ovary cells in a dose-dependent manner [177]. It seems that precise amounts of Sima are needed for the actual border cell migration. It became also clear that Sima was important for the specificity of the leading cells to reside at the edge of their cluster. Changes in the expression of the *DE*-cadherin adhesion protein also implicated Sima [177]. Notably, Sima regulates the activity of the transcription factor slow border cells (Slbo) that is necessary in border cells for their migration [177, 178]. Changes in HIF expression and activity in just a single migrating border cell of the cluster can lead to metastasis in this model [177]. Overexpression of *vhl* led to delay, blockage and acceleration of the border cell migration. Overexpression of *fga* exhibited only acceleration of border cell migration. Moreover, *sima* and *tgo* mutants demonstrate a delayed or accelerative behavior for border cell migration. Therefore, HIF1 activity is required for the conservation of invasive dynamics of the cells that migrate [177].

2.4.2. Blood cell differentiation

A recent study dealing with the role of HIF-1α in *Drosophila* blood cells focused on the interactions between Notch and HIF-1α. A specific lineage of *Drosophila* blood cells, the crystal cells, expressed elevated levels of Sima, even in normoxia [179]. Overexpression of *sima* results in expansion of the population of crystal cells, phenocopying the effect of Notch overexpression.

Thus, both molecules act in the same pathway in the lymph gland. Elevated activation of Notch in crystal cells is further increased in a *sima* overexpression background [179] and the full-length Notch (N^{fl}) receptor can be activated by *sima* in a ligand-independent manner. This happens also in hemocytes that also express *sima*. The N^{fl} is sufficient to increase the number of crystal cells. Even though Tgo acts together with Sima, *tgo* mutants did not reduce the numbers of crystal cells, but they led to an increase in their number [179]. In *Drosophila*, nitric oxide (NO) inhibits PHD and thus promotes the stabilization of Sima, whereas upon hypoxic conditions, it leads to reduced induction of HIF [180–182]. Nitric oxide synthase 1 (NOS1) is highly expressed in mature crystal cells, and its knockdown in the lymph gland by RNA interference (RNAi) leads to reduced numbers of crystal cells. $NOS1^{RNAi}$ clones revealed low levels of the Sima protein and were unable to form crystal cells. In conclusion, Sima is necessary for differentiation of crystal cells in the fly lymph gland [179].

2.5. Upstream regulators of HIF-1α/Sima in hypoxia

A genome-wide RNAi screen was deployed in *Drosophila* cells in culture to reveal genes required for the activation of HIF-1α/Sima [183]. More specifically, 30 genes appeared for the first time as candidate regulators of HIF in low oxygen concentration conditions, and these specific genes mediated the alteration to oxygen starvation. These genes included transcription elongation factors, translation regulators and components of chromatin remodeling complexes [183]. The *ago1* (*argonaute 1*) gene, which has a critical role in microRNA silencing processes, was also identified in this screen as a regulator of Sima in hypoxia. Given the involvement of Ago1 in Sima regulation, the authors went further to show that the microRNA pathway has a central role in HIF-dependent transcription, and also that Sima mRNA stabilization has a critical role in the *Drosophila* response to hypoxia [183].

Further work on Sima regulators has uncovered several modifiers of Sima function in hypoxia in *Drosophila*, such as the microRNA miR-190, the TIP60 chromatin remodeling complex and the RNA-binding protein Musashi [184–186] (**Figure 2**). miR-190 acts as a positive regulator of the hypoxic response by targeting directly the propyl-4-hydroxylase Fga, which is the principal negative regulator of Sima. Specifically, miR-190 is upregulated in hypoxia, reduces Fga activity and, thus, allows the Sima-mediated response to hypoxia [184]. In addition, the TIP60 complex is required for HIF1-dependent gene expression in fly cells and embryos, as well as colorectal cancer cells. TIP60 is recruited by HIF1 to chromatin during hypoxia and functions as a coactivator of HIF1 action by recruiting RNA-Polymerase II onto chromatin [186]. Finally, Musashi (Msi, dMsi in *Drosophila*) represses *sima* mRNA translation by binding an Msi-binding element within the 3′ UTR of the *sima* transcript. dMsi protein levels are reduced in hypoxia, allowing Sima transcription. Thus, dMsi mediates translational repression of the *Drosophila* HIF-1α, Sima. Moreover, association of murine Msi with the HIF-1α transcript suggests that a similar mechanism might be conserved in mammals [185].

2.6. HIF-1α/Sima in *Drosophila* tumorigenesis

The connection between metabolism deregulation and tumorigenesis is already established [187] and various signaling pathways with crucial roles in cancer progression regulate the

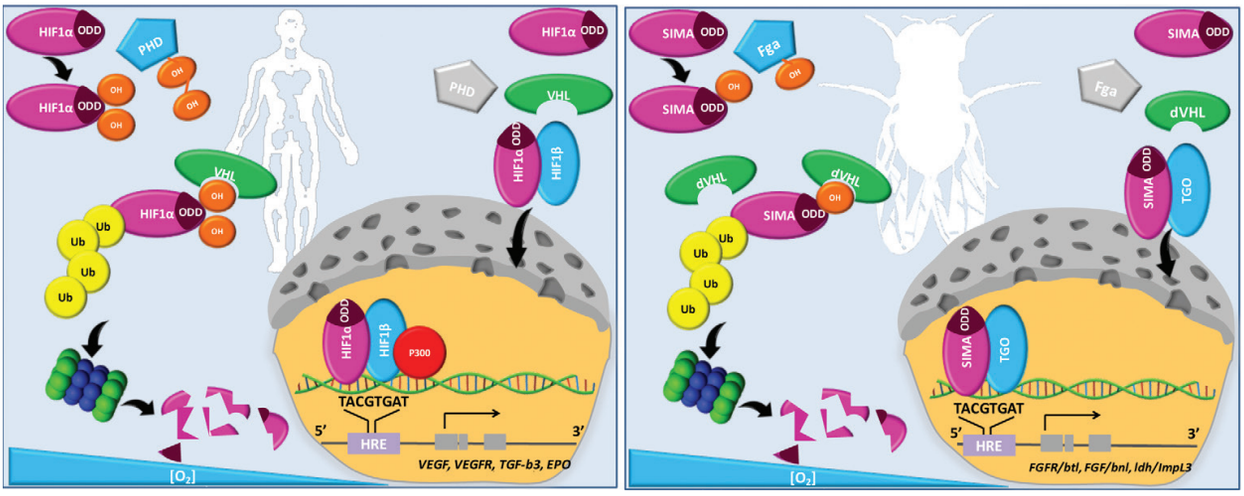

Figure 2. The HIF-1α signaling pathway is conserved between *Drosophila* and mammals. In normoxia, the PHD (Fga in flies) catalyzes the hydroxylation of HIF-1α (Sima in flies). Binding of the hydroxylated HIF-1α by the tumor suppressor protein VHL (dVHL in flies) leads to HIF-1α/Sima polyubiquitination and degradation by the proteasome. In hypoxia, HIF-1α/Sima is stabilized and dimerizes with HIF-1β (Tgo in flies) forming the HIF1 heterodimer. HIF1 is translocated to the nucleus, binds HREs, and induces the transcription of hypoxia-responsive genes.

expression of metabolic genes encoding key glycolytic enzymes [78, 79, 188]. In addition, the transcription factor HIF-1α controls expression of a number of genes involved in different hallmarks of cancer including modifiers of cellular metabolism that facilitate neoplasia [189]. A recent study in *Drosophila* investigated the mechanisms impinging on metabolism deregulation under sufficient oxygen levels and the role of HIF-1α in this process [145] (**Figure 3**). The authors induced a glycolytic tumor in the fly wing imaginal disk by expressing an activated form of the PDGF/VEGF-receptor (Pvr) oncogene, and they observed whether various oncogenic signaling pathways can interact with HIF-1α/*sima* and successively upregulate key metabolic enzymes [145]. Pvr was found to induce strong lactate dehydrogenase (LDH) enzymatic activity, which is a hallmark of aerobic glycolysis and the Warburg effect. Pvr^{act} expression along the anterior-posterior boundary of the wing disk caused extensive growth and dysplasia, and the cells within the mass of this tumor expressed LDH. Thus, the activation of Pvr resulted in *ldh* upregulation, which led to increased LDH enzymatic activity in the tumor. Since Pvr is an RTK, the authors asked if other activated RTKs that lead to overproliferation may also cause increased LDH activity. Interestingly, they found that, unlike Pvr^{act}, InR^{act} and $Egfr^{act}$ did not induce any LDH expression [145]. Sima, one of the key inducers of LDH in tumors [189], was assessed next, and it was found that Sima protein was stabilized in a Pvr^{act} background and its overexpression led to LDH-induced activity [145]. When silencing *sima* in the same background, LDH expression was suppressed. Thus, Sima is necessary for the activation of LDH. Ras^{act}, an effector of all RTKs, was also sufficient to increase LDH activity. Blocking PI3K signaling by targeting different components of the signaling cascade (PI3K, Akt and TOR) led to the suppression of the *LDH-GFP* reporter proving that the PI3K/Akt/TOR axis is necessary for LDH regulation. Co-expression of a gain-of-function allele of human Raf ($hRaf^{act}$) and activated PI3K ($PI3K^{act}$) led to extensive LDH activation, whereas

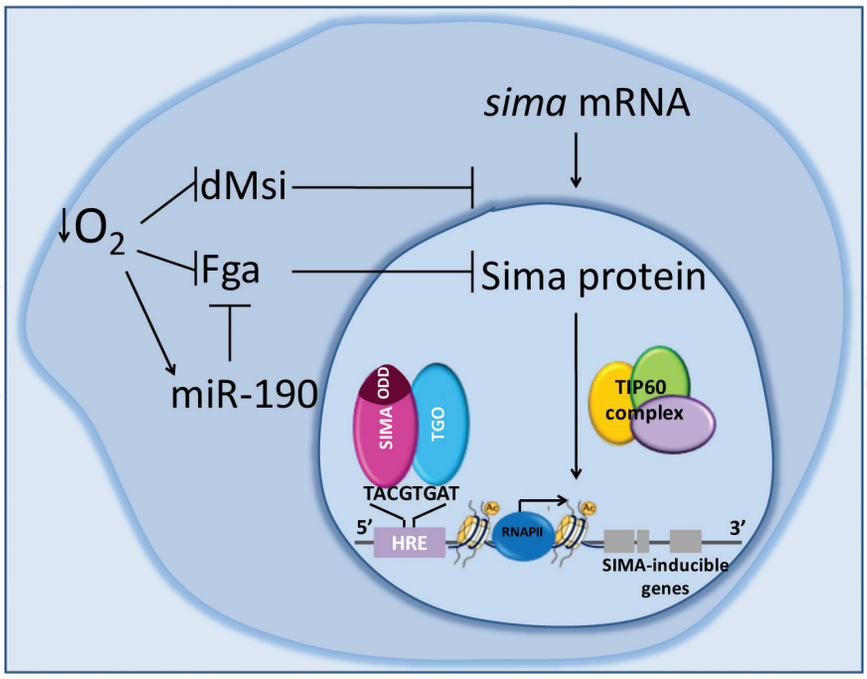

Figure 3. Sima activity is regulated at different levels. In hypoxia, the propyl-4-hydroxylase Fga regulates Sima protein levels post-translationally by promoting its degradation by the proteasome, whereas the RNA-binding protein dMsi represses *sima* mRNA translation. miR-190 is upregulated in hypoxia and inhibits Fga directly, which, in turn, downregulates Sima. Binding of the Sima/Tgo HIF1 heterodimer on the HREs of promoters of hypoxia-responsive genes is necessary for recruitment of the TIP60 chromatin remodeling complex, which, in turn, promotes recruitment of RNA-PolII and efficient transcription.

PI3K/ERK activation led to extensive Sima expression. In the absence of Sima, the PI3K/ERK LDH-GFP expression was reduced. Thus, PI3K and ERK together are necessary and sufficient to induce LDH expression; each signal alone is necessary, but not sufficient, to induce LDH expression. Another known factor that stabilizes HIF-1α is the secretion of ROS even in the presence of sufficient oxygen levels [190]. Peroxidasin (Pxn), a general antioxidant, reduced ROS activity, and when it was expressed together with Pvr^{act} led to the deactivation of the JNK pathway (previously active in a Pvr^{act} background), the suppression of Sima protein accumulation and the reduction of LDH activity. Thus, ROS have a crucial role in metabolism regulation [145]. Moreover, ROS are the central players in the metabolic profile of the cell [191]. They can activate the JNK signaling pathway and use the same mechanisms as hypoxia to stabilize HIF-1α [190, 192]. ROS use a positive feedback loop to strengthen the upstream members of the glycolytic pathway, which is the key point in the metabolic reprogramming of cancer tissues, not only in the absence of sufficient oxygen tensions. Even though hypoxia stabilizes HIF-1α, in the metabolic reprogramming of the cell, hypoxia, unlike HIF-1α, does not play an important role [145]. Interestingly in this study, although Pvr activation was linked to many tumor phenotypes, such as cell shape changes, overproliferation, aerobic glycolysis and local migration, it did not possess any metastatic capability, which correlated with no loss of the cell epithelial polarity that leads to invasive and metastatic abilities [193].

An independent study also assessed Sima expression, as well as induction of tracheogenesis in *Drosophila* epithelial tumors [194] (**Figure 3**). In *Drosophila*, genes involved in the maintenance of apicobasal polarity, such as *lethal giant larvae* (*lgl*), *discs large* (*dlg*) and *scribble* (*scrib*), have been shown to also regulate growth and act as tumor suppressors [195]. Tumors caused by expression of an *lgl* knockdown (lgl^{KD}) in a Minute background in the wing disc of *Drosophila melanogaster* revealed a conserved response to hypoxic stress and also migratory and tracheogenic behaviors [194]. In addition, tumors generated when a loss-of-function mutation of the gene $l(2)gl^{4}$ was combined with the oncogenic form of *Ras*, Ras^{V12}, expressed *bnl*/*FGF* and formed new trachea-like branched structures. Strikingly, cells within the tumor expressed the gene *trh*, a paralogue of *sima*, which is necessary for *btl*/*FGFR* expression and serves as a classic tracheal cell marker in *Drosophila* [135, 196]. These tumor cells induced extra branching of the trachea that was associated with the tumor (similar to sprouting angiogenesis), they synthesized de novo new patterns of trachea-like branches (similar to vascular mimicry), and they migrated toward and incorporated into neighboring pre-existing tracheal tubes (similar to vascular cooption). Importantly, expression of *trh* in the tumor cells transformed some of them to tracheal cells, giving them a different cellular identity and contributing to tumor heterogeneity [194]. Sima exhibited nuclear localization in some of the tumor cells, and this led to activation of the *bnl*/*FGF* promoter. Furthermore, the Polycomb group (PcG) of proteins, which are known epigenetic regulators leading to transcriptional repression in *Drosophila*, was downregulated in the undifferentiated cancer cells, whereas when these cells turned into tracheal cells, they started expressing PcG. This is in agreement with the known function of PcG, which is lowly expressed in stem cells/undifferentiated cells and is upregulated in differentiated cells to lock a particular cell fate [197]. During this epithelial-to-tracheal switch, the JAK/STAT signaling pathway activity was also observed. A correlation between PcG repression and JNK activity was found, whereas the inhibition of JNK led to the opposite result [194]. This publication characterized tracheogenesis of *Drosophila melanogaster* as a novel hallmark of cancer reminiscent of tumor angiogenesis, establishing this model organism as a powerful and promising model for the study of the molecular alterations in the hypoxic microenvironment of the tumor.

3. Conclusions and future perspectives

This book chapter discusses our current knowledge on HIFs and their major roles in development, physiology and disease pathology, using examples of studies in the model organism *Drosophila melanogaster*. The mammalian HIF-1α transcription factor regulates a plethora of genes that promote various aspects of cancer, such as metabolism, invasive motility, growth, angiogenesis and drug resistance (**Table 1**) [189, 198, 199].

The HIF-1α transcription factor has been extensively studied in mammals and in a variety of model organisms due to its highly conserved sequence and function (**Figure 1**). The extensive literature on the *Drosophila* HIF1 pathway suggests that the fruit fly is a potentially good model to study the basic mechanisms of HIF-1α/Sima regulation (**Figure 2**) and identify novel members of the pathway in normoxia and hypoxia (see above). Indeed, a series of studies in *Drosophila* have identified novel

regulators of HIF-1α, which are conserved in mammals and potentially could function in a similar way on mammalian HIF1 [169, 184, 185, 200] (**Figure 3**). Strikingly, recent studies in *Drosophila* [145, 194] underscore the key role of HIF-1α/Sima in tumorigenesis and tumor angiogenesis and suggest that the fruit fly can serve as a great model for studies of the Warburg effect and pathological angiogenesis (**Figure 4**). Impressively, expression of a single oncogene, the activated Pvr, in *Drosophila* can cause glycolytic epithelial tumors that turn on HIF-1α/Sima expression and the glycolysis pathway at the expense of oxidative phosphorylation [145]. Moreover, epithelial neoplasias caused by inactivation of tumor suppressor genes, such as *lgl*, in combination with oncogenic Ras are heterogeneous, and some cells in the tumor induce expression of *HIF-1α/Sima*, *trh*, and *bnl/FGF*, and a subset of them differentiate into tracheal cells. Therefore, these tumors can promote processes reminiscent of sprouting angiogenesis, vascular cooption and vascular mimicry and indicate that, although *Drosophila* does not possess typical blood vessels, its tracheal system may function similar to mammalian blood vessels in pathological situations.

The contribution of HIF-1α/Sima in epithelial tumorigenesis and tracheogenesis in *Drosophila* is intriguing especially in the light of various studies that show a close proximity of tracheal cells with healthy and tumorous epithelia. For example, metastatic tumor cells have been shown to adhere and move alongside the tracheal network [193, 201] and expansion of the trachea has been observed in a PI3K/Ras glioma model in larval and adult brains [202]. In addition, the adult

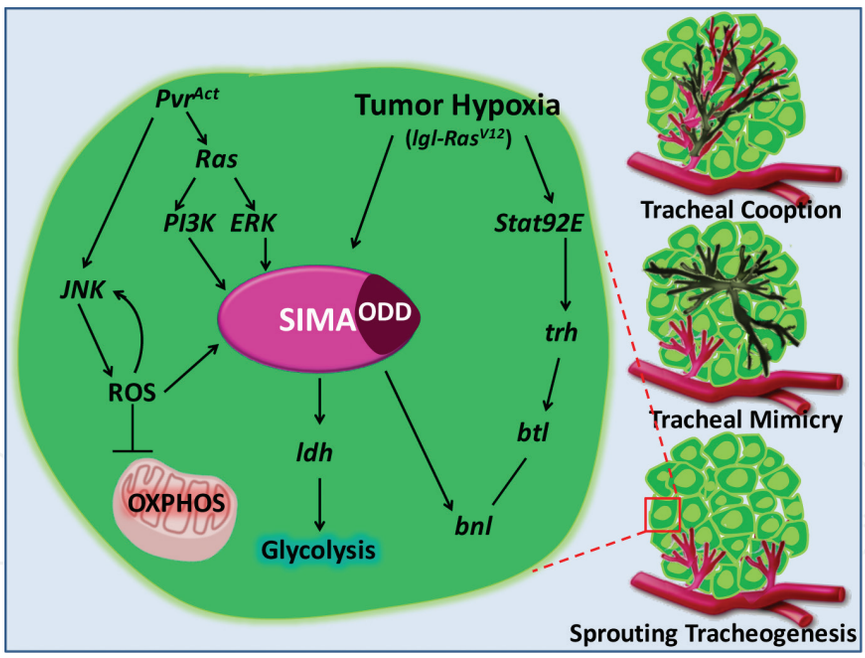

Figure 4. *Drosophila* epithelial tumors induce Sima, the Warburg effect and angiogenesis-like phenotypes. Pvr^{act} triggers the activation of the PI3K/Akt and Raf/ERK signaling pathways in a Ras-dependent manner controlling Sima activity. Pvr^{act} requires JNK activation, which blocks OXPHOS through the production of ROS. ROS produced by the metabolic reprogramming of the tumor responds to the upstream components in order to maintain the Warburg effect. Thus, there is a positive feedback loop that sustains the Warburg effect over time through the activation of LDH, which is induced by Sima. Overall, this mechanism is HIF-dependent but hypoxia-independent. In *lgl-Ras* epithelial neoplasias, tumor hypoxia leads to stabilization of Sima and *bnl/FGF* transcription, as well as STAT92E-dependent *trh* expression, which strengthens activation of the *btl/FGFR* promoter. All these events promote tumor tracheogenesis via three different strategies reminiscent of tumor angiogenesis: sprouting tracheogenesis, tracheal cooption and tracheal mimicry.

fly intestine is oxygenated by an extensive network of visceral trachea that has been shown to contribute growth and regeneration signals to the intestinal stem cells [203–205]. Recently, it has been shown that mutations of the Sox21α transcription factor cause heterogeneous intestinal neoplasias, inside which some tumor cells express *btl/FGFR*, suggesting maybe a phenomenon of tracheal mimicry or cooption [206]. Since many oncogenes and tumor suppressors as well as combinations of mutations with cellular stress caused by pathogens or chemicals can lead to intestinal dysplasia in flies [207, 208], it remains to be seen if and how the HIF-1α/Sima-promoted metabolic changes and tracheogenesis interact with the tumorous environment.

Undoubtedly, *Drosophila melanogaster* has still a lot to contribute toward our understanding of HIF-1α regulation in physiology, hypoxia, tumorigenesis, and angiogenesis. Understanding the function of the HIF pathway in genetically tractable invertebrates will allow the discovery of novel-conserved pathway components that may be used as therapeutic targets in humans.

Abbreviation

ago1	*argonaute 1*
Ago	Archipelago
ARNT2	aryl hydrocarbon receptor nuclear translocator 2
ARNT3	aryl hydrocarbon receptor nuclear translocator 3
ATP	adenosine tri-phosphate
bHLH-PAS	basic-Helix-Loop-Helix-Per/ARNT/Sim
bnl	*branchless*
btl	*breathless*
Cdk4	cyclin-dependent protein kinase 4
CoA	acetyl coenzyme A
CO_2	carbon di oxide
Cul2	cullin 2
dlg	*discs large*
dMsi	*Drosophila* Mushashi
EGFR	epidermal growth factor receptor
EGFs	epidermal growth factors
EPO	erythropoietin
fga	*fatiga*
FGF	fibroblast growth factor
GLUTs	glucose transporters
GLUT1	glucose transporter 1
GRPs	glucose regulated proteins
HIFs	hypoxia-inducible factors
HIF1	hypoxia-inducible factor 1

HIF-1α	hypoxia-inducible factor-1α
HIF-1β	hypoxia-inducible factor-1β
HIF-2α	hypoxia-inducible factor-2α
HIF-3α	hypoxia-inducible factor-3α
$hRaf^{act}$	human Raf gain-of-function
HREs	hypoxia-response elements
ID	inhibitory domain
IGF-I	insulin growth factor I
ILP	insulin-like peptide
IL-1α	interleukin-1α
IL-1β	interleukin-1β
IL-8	interleukin-8
InR	insulin receptor
LDH	lactate dehydrogenase
LDHA	lactate dehydrogenase A
lgl	*lethal giant larvae*
lgl^{KD}	*lgl* knockdown
miR	microRNA
Msi	Musashi
NF-κB	nuclear factor-kappa B
N^{fl}	Notch full-length
NLS-C	carboxy-terminal Nuclear Localization Signals
NLS-N	amino-terminal Nuclear Localization Signals
NO	nitric oxide
NOS	nitric oxide synthase
NOS1	nitric oxide synthase1
ORPs	oxygen regulated Proteins
OXPHOS	oxidative phosphorylation
O_2	oxygen
PcG	polycomb group
PDGF-β	platelet-derived growth factor beta
PDK1	pyruvate dehydrogenase kinase 1
PDK-1	phosphoinositide-dependent kinase 1
PHD	prolyl-4-hydroxylase
PI	phosphatidylinositol
PI3K	phosphatidylinositol 3-kinase

$PI3K^{act}$	PI3K activated
PI-3P	phosphatidylinositol 3-phosphate
PKB	protein Kinase B
Pro	proline
PSTD	Proline-Serine–Threonine-rich protein stabilization Domain
PTEN	phosphatase and TENsing homolog
Pvr	PDGF/VEGF-receptor
Pxn	peroxidasin
Rbx1	ring box protein 1
RNAi	RNA interference
ROSs	reactive oxygen species
scrib	*scribble*
sima	*similar*
Slbo	slow border cells
TAD-C	carboxy-terminal transactivation domains
TAD-N	amino-terminal transactivation domains
TGF-α	transforming growth factor alpha
tgo	*tango*
TNF-α	tumor necrosis factor α
trh	*trachealess*
VEGF	vascular endothelial growth factor
VHL	von Hippel-Lindau

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