Recent Advances in Understanding the Molecular Basis of Infantile Haemangioma Development

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What is already known about this topic?

- Angiogenesis and vasculogenesis play critical roles in the formation of Infantile Haemangioma.
- Several signalling pathways such as VEGF, HIF, FGF, PI3K/Akt/mTOR have been identified regulating the development of Infantile Haemangioma.

What does this study add?

- This review provides a summary of all the key pathways identified so far with detailed downstream molecular interactions.
- Hormonal receptors regulate progression of Infantile Haemangioma.

Abstract

Infantile haemangioma (IH), the most common vascular tumour of infancy, is comprised of diverse cell types including endothelial cells, pericytes, fibroblasts and immune cells. IH is characterized by rapid proliferation followed by slow involution over 1 - 10 years. Most lesions regress spontaneously, but up to 10% can be disfiguring with complications that require further medical treatment. Recent research has revealed the biological characteristics of IH, highlighting the involvement of angiogenesis and vasculogenesis during tumour formation. Gene expression profiling has provided vital insights into these underlying biological processes, with some of the key IH-related pathways identified, including VEGF, RAAS, HIF-1α, Notch, PDGF, PI3K/Akt/mTOR, JAK/STAT, FGF, PPARγ, IGF. Further evidence suggests extracellular matrix factors and hormone receptors regulate IH progression. In this review, we explore the molecular mechanisms involved in the proliferating, plateau and involuting phases of IH. This

involves identifying differentially expressed genes, targeted proteins, and key signalling pathways. This knowledge will increase the broader understanding of vascular development, tissue remodelling and angiogenesis.

Overview of IH Pathogenesis

Infantile haemangioma (IH) is the most common benign vascular tumour in infants, affecting 5-10% of newborns[1–4]. It is characterized by excessive proliferation of endothelial cells and presents as a red and sometimes raised lesion on the skin and/or subcutaneous tissue that varies in number, size, shape, and location. IH develops early in life, usually within the first few weeks, and follows a distinctive growth pattern in which the tumour grows rapidly during infancy and then spontaneously involutes over several years[1]. The incidence of IH is higher in female, Caucasian and premature infants[3,5]. Pre-term babies have a 40% increased risk, with 1 in 4 developing IH[6]. Maternal factors such as advanced age, multiple pregnancies, maternal complications, such as, pre-eclampsia and amniocentesis, a history of infertility treatments, and conception through *in vitro* fertilization, may also contribute to an increased incidence of IH[3,7].

IH can be categorized into focal or segmental lesions[3]. Focal IHs are the most common and usually present as a solitary lesion(s) that can affect any part of the body. Segmental IHs are much less common and are distributed in a segmental fashion following the developmental axis, often affecting the face and less commonly the limbs or trunk. Furthermore, multifocal IHs occur in about 3.6% cases, and occurrence of more than five lesions increases the risk of liver involvement (50% cases), with a potential risk of peripheral hypothyroidism and rare intestinal involvement[8]. Segmental IHs are more likely to be

complicated by ulceration leading to functional and cosmetic impairment and often associated with midline structural anomalies in the posterior fossa, coarctation of the aorta, absent or anomalous arteries in the skull base, cardiac and/or eyes anomalies and sternal raphe/cleft (PHACE syndrome)[7].

IH is characterized by rapid proliferation during infancy (proliferating phase) followed by spontaneous slow regression (involuting phase) when the proliferating endothelial cells are gradually replaced with fibrofatty tissue (involuted phase) (Figure.1)[9,10]. During the proliferating phase, complications such as ulceration with pain and bleeding, tissue distortion and functional problems such as visual or airway obstruction, may occur. Several factors may be responsible for this transition, such as immune response, hormone regulation, and interaction with cell signalling pathways.

Most IHs do not require active intervention. The indications for active treatment include a threat to life, (vision, airway obstruction; tissue distortion or ulceration). Large or multiple lesions especially those affecting the liver may cause hypothyroidism and high output cardiac failure[11].

Historically, problematic proliferating IHs were treated with high-dose steroids which leads to accelerated involution in 30% of cases, and stabilization of the lesions in 40% with continued growth of the tumour in the remainder[12]. Complications of steroid administration is well known and including insomnia, hypertension, adrenal suppression, growth retardation, gastrointestinal reflux, and immune suppression[3]. Repeated intralesional steroid injections, usually performed under general anaesthesia had been used for localized IHs and pulsed dye laser treatment have been used for superficial ulcerated lesions[12]. Interferon-α has been used for cases of problematic proliferating IHs refractory to systemic steroids. However, this treatment has been abandoned in view of neurotoxicity such as spastic diplegia,

in favor of the chemotherapeutic drug vincristine which is associated with toxicity such as neutropenia[7,12,13].

The non-selective β-blocker propranolol is the mainstay treatment for problematic proliferating IH since its serendipitous discovery to cause accelerated involution of IH in 2008[14]. Other β-blockers including β1 selective-blocker atenolol, have been used for the treatment of IH[15]. Although an effective treatment in 85-90% of cases[16], there are limited long-term studies investigating the effects of β-blockers on infant development. A study by Tan, Guo and Wang 2021 reported significant adverse side effects of beta-blockers in babies, including hypertension, hypoglycaemia, sleep disturbances and bradycardia[17] . This resulted in some patients ceasing treatment early, which is not optimal as in rare cases it has been associated with treatment resistance or rebound of tumour growth[7]. Sub-therapeutic dosages have sometimes been recommended as this is associated with low rate of side-effects[18]. Another study by Hali *et a*l 2023 focused on predictors of poor response to propranolol, and found that 13.4% patients with multiple or segmental haemangiomas responded poorly, but there was no association with sex or age[19]. Topical β-blockers, such as timolol, are indicated for small, superficial IH but there are limited studies with sufficient patient numbers and limited efficacy has been seen with topical treatment[20]. Although the indications for oral β-blockers for problematic proliferating IHs have broadened from those for systemic high-dose steroids, majority of IHs are not actively treated because of its potential side effects. This demonstrates the need for new IH treatment options including topical remedies for less severe cases. Further research is needed to investigate the long-term effects of propranolol in infants, including the potential adverse effects of system β-blocker administration during infancy on development and growth.

Molecular Mechanisms of Proliferating Infantile Haemangioma

The cells that make up the IH play a unique role in the pattern of tumour growth and regression. These cell types include haemangioma-derived endothelial cells (HemECs), haemangioma-derived stem cells (HemSCs), pericytes, fibroblasts, immune cells[21,22], with adipocytes appearing during the involuting and involuted phases. The rapidly dividing HemECs are the predominant cell type present during the proliferative phase; these plump cells respond to growth factors such as VEGF, forming new immature blood vessels with tiny lumens[23]. They also interact with many other cells, including pericytes and immune cells such as mast cells[24].

Early research investigating the cellular origins of IH predict a population of haematopoietic stem cells or HemSCs as endothelial progenitor cells, which is supported by the detection of cell markers associated with primitive haematopoietic cells in the endothelium of proliferating IH. A possible placental origin is also demonstrated due to similarities in HemEC and placental cellular markers, such as GLUT-1, the diagnostic marker of IH[1,25–27]. GLUT-1 is a unique maker for IH, shared with placenta and it differentiates this tumour from other vascular anomalies[28]. C19MC is the largest miRNA cluster in humans and consists of 46 miRNAs. This cluster is unique to IH compared to other vascular anomalies and is found circulating in serum of IH patients. They are released by the GLUT-1+ ECs, and hence might be a potential biomarker for this tumour[29–31]. HemSCs, which constitute about ~1% of the total cell composition, express the stem-cell marker CD133, and have been shown to be progenitors of HemECs and adipocytes during regression[7,32]. HemSCs also control the interaction of Hem-pericytes that wrap around blood vessels and control HemEC growth, blood vessel lumen size and assembly of extracellular membrane matrix. During early to late involuting stages, immune cells and primarily mast cells are also observed in IH microenvironment[22,33]. These cells have been associated with inflammation and regression of IH[10]. Iterations in molecular processes occurring in these cells predict the phase and progression of IH from

aggressive growth to gradual involution[34,35]. Many molecular pathways are dysregulated in IH, and these are summarized in Table 1.

Pro-angiogenic factors

Vasculogenesis and angiogenesis are key processes in the proliferating phase of IH, with vasculogenesis involving differentiation of angioblasts into endothelial cells and angiogenesis involving the growth of new capillaries. The proliferation and migration of endothelial cells that initiate angiogenesis, is regulated by pro-angiogenic factors such as VEGFR, ANGPT2 and HIF-1α, which activate the endothelial cells to sprout and form tubular structures, creating a network of new microvessels. The rapid vasculogenesis gives rise to hypoxic regions that function as a positive feedback loop for further blood vessel formation[36–39]. VEGF receptors regulate angiogenesis, promoting and maintaining blood vessel growth. Two receptors of the VEGF family are expressed in HemECs: VEGFR1 and VEGFR2. VEGFR-1 has higher binding affinity for its ligand than VEGFR-2, but weaker kinase activity. Endothelial cells expressing high levels of VEGFR2 show superior growth[34]. Decreased VEGFR1 expression is seen in proliferating HemECs, due to its role in suppressing angiogenesis. However, VEGFR1 expression is increased in HemSCs, leading to their differentiation through the activation of VEGFR1 by VEGF-A. HemECs are then stimulated by VEGFR2 to undergo immature vessel development (Figure.2). Consistent Bcl-2 expression prevents apoptosis of HemEC through the constitutively active autocrine VEGF-A/VEGFR2 loop, further promoting growth during the proliferative phase of IH. Other downstream pathways activated by VEGF include MEK/ERK, PI3K/Akt, PKC, which regulate survival and migration of cells, and therefore, may further amplify the growth of IH[34,38].

Hypoxia has been proposed to play a role in the pathogenesis of IH[37]. One of the most potent stimulators of vasculogenesis is hypoxia. Tumour cells become hypoxic when they exceed the maximum

diffusion distance from nearby blood vessels and the angiogenic balance becomes dysregulated, leading to a release of growth factors such as VEGF, FGF, PDGF among others[16]. The transcription factor hypoxia-inducible factor-1α (HIF-1α) is expressed at a higher level on HemECs than in normal endothelial cells, leading to upregulated VEGF and GLUT-1 expression. Propranolol suppresses survival of HemEC by targeting this HIF-1α/VEGF pathway[4,34].

The renin-angiotensin system (RAAS) is an endocrine system that maintains blood pressure and body fluid homeostasis, which can be dysregulated in disease states[34]. Prorenin receptor (PRR), a transmembrane protein, binds precursor prorenin and active renin. PRR is present on IH and is localized to the non-endothelial and the endothelial IH cell populations[40]. Renin, a glycoproteolytic enzyme catalyses angiotensinogen into angiotensin I. Angiopoietin II (ANGPT2) is the downstream vasoactive peptide responsible for cell proliferation, via angiotensin II receptor 2 (ATIIR2) and angiotensin II receptor 1 (ATIIR1). The components of RAAS, PRR, angiotensin II and ATIIR2 and angiotensin converting enzyme and ANGPT2 are also expressed in the endothelium of IH and appear to play a role in pathogenesis[41]. Angiotensin II causes cellular proliferation in IH via ATIIR2 activation[42]. Serum levels of renin, angiotensin converting enzyme and angiotensin II decreases in patients treated by surgery, propranolol and captopril for problematic proliferating IH[43] . Furthermore, renin promotes proliferation of HemECs and inhibits the canonical Wnt signalling pathway, therefore increasing angiogenesis during the proliferation phase[40,44] (Table.1). One of the mechanisms by which β-blockers may induce accelerated involution of IH is by targeting the RAS pathway, which is upregulated during the proliferating phase of IH[18].

Signalling pathways promoting cellular proliferation in infantile haemangioma

Aberrant cell proliferation in IH is induced by FGFR, PI3K/Akt/mTOR, JAK/STAT and PDGF signalling pathways. Overexpression of fibroblast growth factor (FGF-2, also known as basic fibroblast growth factor, bFGF) occurs during the proliferative phase of IH, and is downregulated during the involuting and involuted phases. Furthermore, FGF-2 is a specific angiogenic factor that stimulates endothelial cell proliferation and promotes fibroblast migration as well as generation of collagen, fibronectin, and integrin (Table.1). FGF-2 binds to the fibroblast growth factor receptor 1 (FGFR1) and inhibits ERK1/2 phosphorylation and PI3K stimulation. This subsequently initiates downstream signalling of mTOR[23,39]. HemECs have an active P13K/Akt/mTOR pathway, which is inhibited by rapamycin, resulting in low HIF-1α and VEGF-A expression[10]. *In vivo* studies have demonstrated that rapamycin decreases the proliferation, differentiation of HemSCs, although this treatment in infants is associated with side effects[34]. miR-126 and miR-210 are pro-angiogenic and they stimulate HemEC proliferation by *b*FGF and VEGF signalling[54,55].

Phosphorylated and activated forms of STAT1, STAT3 and STAT5 of the JAK/STAT signalling pathway has been reported to be expressed in the endothelium of IH and cells within the interstitium in the proliferating phase with reduced expression in involuted IH. Angiotensin II receptor 2 induces STAT3, hence influencing IH growth using RAAS pathway[45]. The platelet derived growth factor family (PDGF) comprises ligands PDGF-A, -B, -C, and -D, which bind to the tyrosine kinase receptors, PDGFR-α and PDGFR-β. Activation of PDGF signalling results in altered cell survival and angiogenesis. PDGF signalling inhibits differentiation of HemSCs to adipocytes in proliferating IH and hence its expression is lower

during involution when adipogenesis is high[46]. HemSCs also give rise to Hem-pericytes, which surround HemECs controlling differentiation and proliferation[32]. Moreover, the endothelium is an important source for PDGFR β for mural cell enrichment, and blocking PDGFR β leads to increased vessel width and a decrease in basement membrane matrix deposition. PDGFR levels are elevated during the proliferation phase and inhibit involution, although, their role in the pathogenesis of IH is unclear[34].

Matrix metalloproteinases are a family of protein and peptide hydrolase that includes MMP-1, MMP-2, MMP-3, MMP-9, and MMP-10. They play a crucial role in extracellular matrix (ECM) degradation by hydrolysing basement membrane collagen. MMP-2 and MMP-9 are activated by growth factors, growth factor binding proteins, and cytokines, to breakdown collagen IV, V and X. MMP-9 is a metal-ion dependent protease and can progress vessel development by VEGF regulation (Figure.2). Overexpression of MMP-2 occurs in the cytoplasm and ECM of the proliferating endothelial cells in IH. MMP-2 is a type IV collagen that lyses gelatin, elastin and collagen IV, V, VII, IX, X, all of which promote EC migration and the formation of a capillary network[38,56]. The proliferative phase of IH is characterized by an aggressive growth of the tumour with rapid formation of a vascular network, followed by spontaneous slow involution. Deciphering the cellular mechanisms that occur during the proliferative and involution phases of IH needs a deeper understanding of the aetiology of this tumour, which may elucidate potential factors involved in triggering subsequent involution.

Molecular Changes during Involution of Infantile Haemangioma

Anti-angiogenic and pro-angiogenic factors

Anti-angiogenic pathways including tissue inhibitor of metalloproteinase (TIMP) and Notch are upregulated in involuting IH[34,56]. TIMPs suppress the activation of pro-MMPs thereby inhibiting ECM

degradation and angiogenesis (Figure.2). They have multiple functions including promotion of fibroblast proliferation and collagen synthesis. They also induce development of fibrous tissue and can sometimes lead to the necrosis of ECs. Interestingly, overexpression of TIMPs is observed in degenerated vascular ECs and the cells of connected tissue but not during the proliferative phase of IH[56].

The Notch signalling pathway is a vital cascade in IH. It encompasses both pro-angiogenic and anti-angiogenic factors, playing a pivotal role in balancing the angiogenic switch, which can influence the progression and involution of these tumours. Notch receptors (Notch 1 to 4) interact with ligands (Delta-like 1, -3, -4, Jagged-1, -2) that cleave transmembrane Notch receptor, resulting in the migration of the Notch intracellular domain (NICD) to the nucleus. This leads to overexpression of Notch target genes, the hairy and enhancer of split (HES) and HES-related protein (HERP/HEY) family of transcription factors[34]. Components of the Notch signalling pathway have different effects on HemECs, pericytes and HemSCs. Notch-3, HES1, HEY1, and HEYL are overexpressed in HemSCs, while Notch-1, -4, Jagged-1, and HEY2 levels (though not uniformly expressed) are high in HemECs[34,57] (Table.1). Interestingly, some studies have reported Jagged-1 expression in Hem-ECs results in mural cell differentiation through Notch-3 activation in HemSCs during involution. This signalling inhibits blood vessel formation in murine models, and such activation in pericytes reduces cell proliferation and cell cycle arrest[48].

Laser capture microdissection and genome-wide transcriptional profiling[47] show, high NOTCH-4 and Jagged-1 expression in the proliferative phase of IH when compared to placenta. Imbalance of Jagged-1 and DLL4 expressions is more prevalent in HemECs than HemSCs. Jag2 mRNA and protein are highly expressed in proliferating IH than involuting lesions, with no Dll3 expression [48]. Interestingly, Dll3 loss leads to increased Notch signalling. The crosstalk between Notch and its ligand pairs specifies ECs to stalk and tip cells[34]. Dll4 and Notch-1 activation confers stalk cell identity in ECs, which is inhibited by

Jagged-1. On the other hand, Dll1 interacts with Jagged-1 to antagonize vascular development. VEGF and Notch pathways have been found to interact with each other in several studies, although with contradictory findings on the regulation of these pathways in IH. VEGF increases DII4 expression, although VEGFR-2 expression decreases Notch and VEGFR-1 activity, which drives proliferation of ECs[34,48]. More research is needed to elucidate the subtle changes in gene expression and molecular adaptations within these pathways pre- and post- phase transition to further our understanding of the progression of IH (Figure 2.)

Apoptosis and adipogenesis

The involuting phase of IH starts with a simultaneous increase in apoptosis and onset of adipogenesis[23,49], resulting in regression of blood vessels and tumour shrinkage, although the trigger is not yet clearly established. The nuclear receptor peroxisome-proliferator activated receptor (PPARy) promotes a pro-adipogenic and anti-inflammatory response during involution and can interact with estrogen to regulate adipogenesis[32]. Perivascular mesenchymal stem cells, also known as pericytes, regulate adipogenesis in IH by the PPAR-y pathway and differentiate into adipocytes during the involuting phase[50]. During the involuting phase, there is an increase in the expression of PPARy2, LPL, CEBPα and Peripilin A that is associated with the differentiation of IH mesenchymal stem cells to adipocytes[49] (Table.1). This suggests the PPARy pathway plays a vital role in suppressing angiogenesis and increased apoptosis of HemECs in the involuting phase[49] (Figure.2). COX-2 inhibition has also been hypothesized to be correlated to the PPAR-y/CEBP signalling pathway to promote involution[52]. In another study, expression levels of CEBPα, CEBPβ, PPARy and adiponectin are increased in HemSC during adipogenesis when treated with DAPT (N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester), an inhibitor of the Notch pathway[58].

The Insulin-growth factor (IGF-1) signalling pathway is also important for cell proliferation and differentiation and has a crucial role in adipogenesis and activation of HemSCs, via the IGF-1 receptor and downstream PI3K signalling pathway[53]. IGF-2 also induces HemSC adipogenic differentiation through the same mechanism[51,52]. The characterization of mechanisms driving adipogenesis and apoptosis in IH will shed light on the natural progression of IH, particularly the proliferative and involuting phases, and development of therapies targeting the factors that trigger involution.

Hormonal influence on Infantile Haemangioma

IHs are more common in females compared to males, with research showing a female-to-male ratio up to 3:1[3,5]. Sex-based differences in anatomical location of IH have also been reported, with a higher prevalence of IH on the head and neck in females, and a higher prevalence on the trunk and extremities in males. These distinctions extend to the timing, rate of growth, and the eventual involution process, which can vary between females and males. Moreover, females may be more prone to developing multiple IHs, whereas males may present with larger, single lesions[59].

Sex hormones, particularly oestrogen, have been implicated in the development and progression of IH and in part, may explain the increased prevalence in females. Growing evidence suggests a connection between oestrogen and IH, with one study reporting increased serum oestradiol levels in IH patients compared to healthy children[60]. Additionally, it has been shown that oestrogen and VEGF synergistically increase the proliferation of HemECs *in vitro*[61]. However, the role of oestrogen and its receptors in the pathogenesis of IH remains poorly understood. A recent study by Johnson *et al* 2021 reports a diverse expression of sex hormone receptors including oestrogen receptor (ER) and progesterone receptor (PR) during the development of IH[62]. There are three major forms of oestrogens; oestrone (E1), oestradiol

(E2, or 17β-oestradiol) and oestriol (E3), with E2 being the most widely studied. Oestrogen acts through two types of receptors, nuclear receptors (ER-α and ER-β) and cell membrane receptors (GPR30 & ER-X). The nuclear receptors are ligand-regulated transcription factors that control the expression of genes required for development of the reproductive system. E2 and ERα are highly expressed in mural cells in IH, but not in ECs. Mural cell precursors are attracted to the tumour by E2 and can migrate into the IH tissue and mature, and once activated, they secrete pro-angiogenic (FGF-2) and anti-angiogenic factors (IFN-α, IFN-β, IFN-γ, TGF-β) in the tumour microenvironment, which are taken up by the adjacent cells including fibroblasts, plasma cells and macrophages and may be involved in involution of IH (Figure.3) [60]. ERα & ERβ are present on adipocytes and their expression can alter during differentiation, indicating these cell types may be regulated by hormones (Figure.3). PRs also have similar roles of reproductive function and gene transcription in cell growth and survival[63,64]. ERα and PR mRNA and protein levels also increase during involution of IH compared to the proliferative phase, although ERβ mRNA do not relate to the protein expression due to post-transcriptional modifications[62].

Surprisingly, oestrogen levels are higher in newborns with IH, and this is associated with higher VEGF expression. Limited studies have shown tamoxifen, a selective ER modulator commonly used for the treatment of breast cancer, to lower VEGF expression in an ER-dependent manner[65]. PR receptors are most highly expressed in IH among all hormone receptors, although research on the significance of this expression in IH is lacking, raising the possibility that using hormone therapies during pregnancy will raise the risk of IH[62]. The circulating levels of oestrogen and progesterone in newborns drastically decrease in the first month after birth, and then suddenly increase during the first year of life followed by gradual decline. This corelates with the progression of IH (Figure.4).

The gonadotropin follicle-stimulating hormone (FSH) is also associated with the progression of IH[4,66]. Notably there is higher expression of the FSH receptor in the proliferative phase compared to the involuting phase. Clinically, the levels of FSH are higher in females than males, and are 40% higher in low birth weight and pre-term newborns, correlating to the incidence of IH with increased FSH levels within 12 months of life (Figure.4)[67,68]. FSH promotes HemSC proliferation and cell-cycle progression (G1/S phase transition)[69], while another study shows that it inhibits apoptosis by stimulating protein kinase B (PKB/Akt)[70], upregulating VEGFR2 and anti-apoptotic Bcl-2 expression, which taken together, further implicate this sex hormone in the pathogenesis of IH.

Further research is needed to elucidate the influence of sex hormones on progression and involution of IH and elucidate its higher prevalence in females. It is possible that subtle variations in gene expression and/or hormonal response and subsequent metabolic processes could confer protective effects in males vs females. Physiological cues and differential gene expression in males and females could potentially expedite or slow down the transition from proliferative phase to involuting in IH. Similarly, responses to treatment, such as propranolol, may be altered between sexes and therefore, a more targeted approach may be warranted when considering sex differences in the treatment of IH.

Conclusions and Future perspectives

A comprehensive understanding of the pathogenesis and the nuanced pathway changes that govern during the programmed biological behaviour of IH is crucial for the development of more targeted treatment approaches. The proliferative phase of IH is associated with high angiogenesis and vasculogenesis, and upregulation of signalling pathways promoting cell survival. Several genes that are significantly upregulated in IH are implicated in the VEGF, FGF, PI3K/Akt/mTOR, RAAS, HIf-1α, Notch,

PDGF pathways [23,34]. Decreased angiogenesis with subsequent adipogenesis is important for the progression into the involuting phase. Stopping or reducing IH growth by targeting HemSCs and promoting regression has been a challenge that the research field must address. By unravelling the intricacies involved in these gene mutations, improving the management of IH, reducing side-effects, and improving quality of life. There is scope for advancing haemangioma research and treatment in terms of topical vs oral, genomic, and proteomic profiling and investigating the role of diverse types of cells involved in the progression of IH. Therefore, stage and sex may be crucial factors in the development of future treatments but requires further research to fully understand the molecular mechanisms of IH. There remains a significant gap in translating research from bench to bedside due to a lack of accurate models that replicate the complexity and tumour microenvironment of IH, along with insufficient characterization of haemangioma progression. Only through addressing these hurdles can a more seamless translation of IH research into new and effective strategies for treating infants with IH be achieved.

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Figure legends

Figure 1. Overview of the phases (proliferating, plateau and involuting) in the progression of Infantile Haemangioma along the course of time. IH starts with the proliferating phase within the first 12 months of life after birth, and then plateau or quiescent phase for 12-18 months, followed by the involuting phase for up to 10 yrs (Created with BioRender.com).

Figure 2. Molecular mechanisms involved in the pathogenesis of Infantile Haemangioma (IH). Haemangioma Stem-cells (HemSCs), endothelial cells (HemECs) and pericytes (Hem-pericytes) play different roles in the proliferating and involuting phases. HemECs actively divide and form blood vessels due to upregulated VEGF, Notch, FGF2, TIE/RAAS, JAK/STAT, PI3K/Akt/mTOR signalling pathways. HemSCs stimulate HemECs via the VEGF and TIE2 pathway and Hem-pericytes prevent adipogenesis (PDGF-β pathway). In the involuting phase, adipogenic differentiation of HemSCs (PPARγ, Notch pathway) and apoptosis of HemECs (downregulation of angiogenic factors MMPs by TIMPs, and other proliferative signalling pathways) occurs (Created with BioRender.com).

Figure 3. Oestrogen hormone regulation in IH during the proliferating and involuting phase in the pericytes and adipocytes, respectively. Both pro- (FGF2, VEGF) and anti-angiogenic (IFN-α, IFN-β, IFN-γ) factors are released in pericytes, whereas oestrogen activation leads to differentiation of adipocytes during regression (Created with BioRender.com).

Figure 4. Changes in sex hormone levels in the first year of life in infants correlated to occurrence of IH (proliferating phase). Infantile Haemangioma occurrence has been found to be associated with the increase in hormonal levels by first 12 months post birth, also known as mini puberty (Created with BioRender.com).

Table 1. Summary of dysregulated signalling pathways in Infantile Haemangioma

Signalling pathway	Components	Interactions	References
VEGF	VEGFR1 VEGFR2	VEGFR1 suppresses angiogenesis in HemECs & suppresses differentiation of HemSCs to HemECs; VEGFR2 initiates immature vessel development	[34,38]
HIF	HIF-1α	HIF-1α over-expression in HemECs lead to increased VEGF & GLUT-1	[7,16,34,37]
RAAS	ANGPT-2 PRR ACE ATII ATIIR2	Renin promotes HemEC proliferation, ANGPT-2 induces HemSC growth; inhibit Wnt signalling pathway	[34,40,44]
FGF	FGF2	HemEC growth, fibroblast migration, ECM generation, ERK1/2 phosphorylation, activate PI3K/Akt/mTOR pathway	[23,39]
JAK/STAT	STAT1 STAT3 STAT5	pSTAT1, pSTAT3, pSTAT5 expressed in HemEC & cells within the interstitium in proliferating IH	[45]
PDGF	PDGFR-β	PDGFR-β inhibits differentiation of HemSC to adipocytes; Hem-pericytes express PDGFR-β (role unknown)	[32,34,46]
Notch	Notch-1 Notch-3 Notch-4 HES1 HEY1 HEY2 HEYL Jagged-1	Notch-3, HES1, HEY1, HEYL are overexpressed in HemSCs, while Notch-1, -4, Jagged-1, HEY2 (though not uniformly expressed) levels are high in HemEC; Jagged-1 differentiates mural cells through Notch-3 activation in HemSCs during involution; Dll4 & Notch-1 activates stalk-cell conformation in HemEC	[34,47,48]
PPARy	PPARy2 CEBPa CEBPb LPL Peripilin A	adipogenesis in HemSC, HemEC apoptosis, suppress angiogenesis	[49,50]
IGF	IGF-1 IGF-2	adipogenesis in HemSC via PI3K pathway	[51–53]

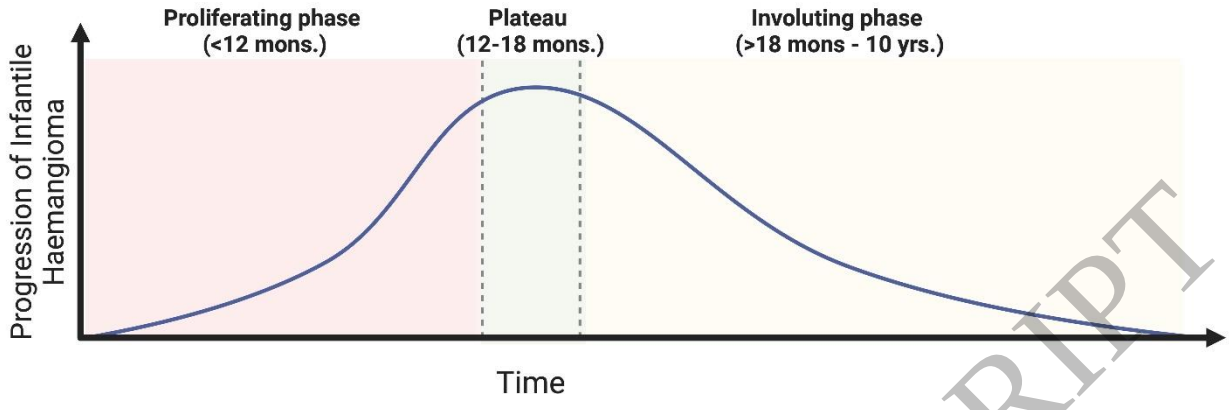

Figure 1
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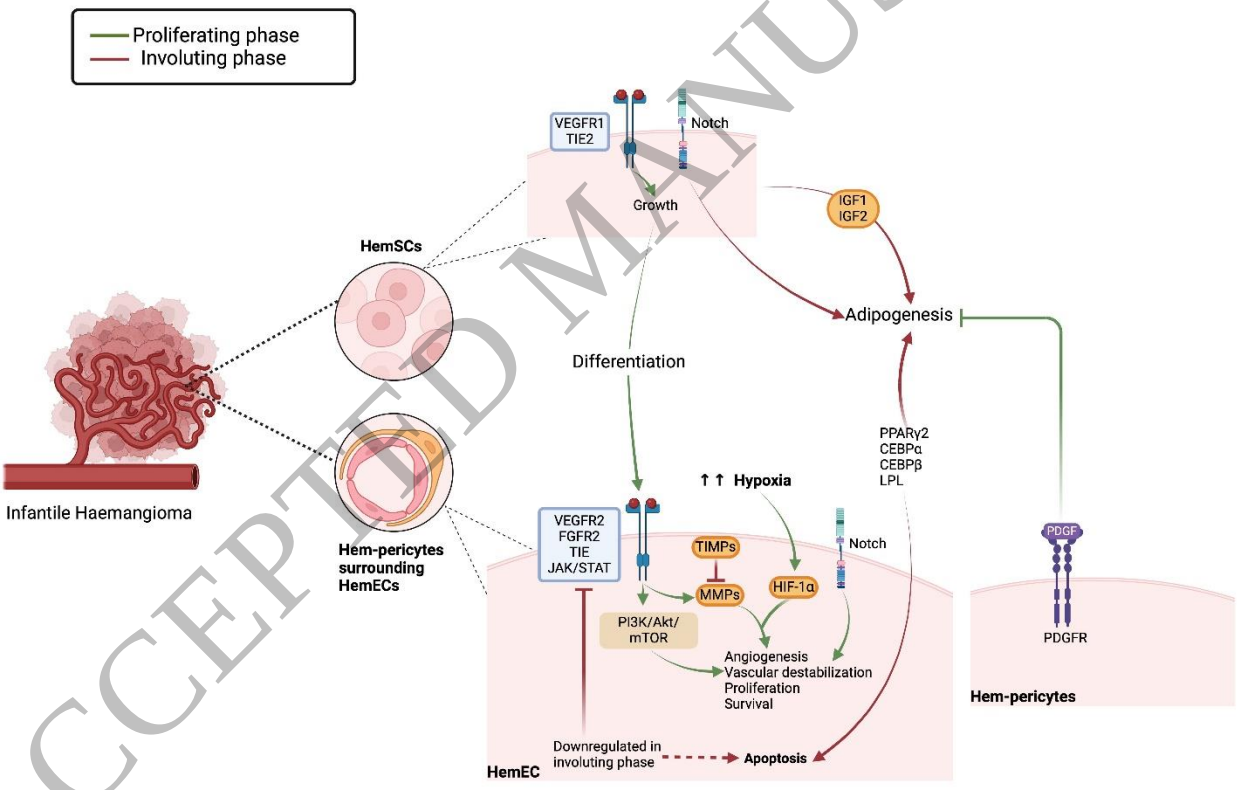

Figure 2
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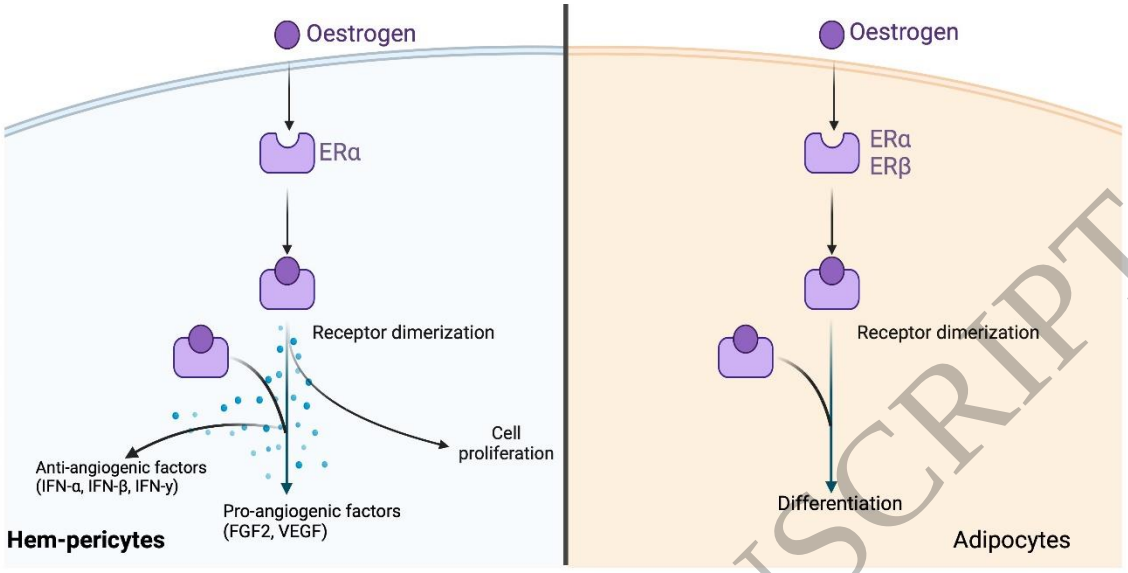

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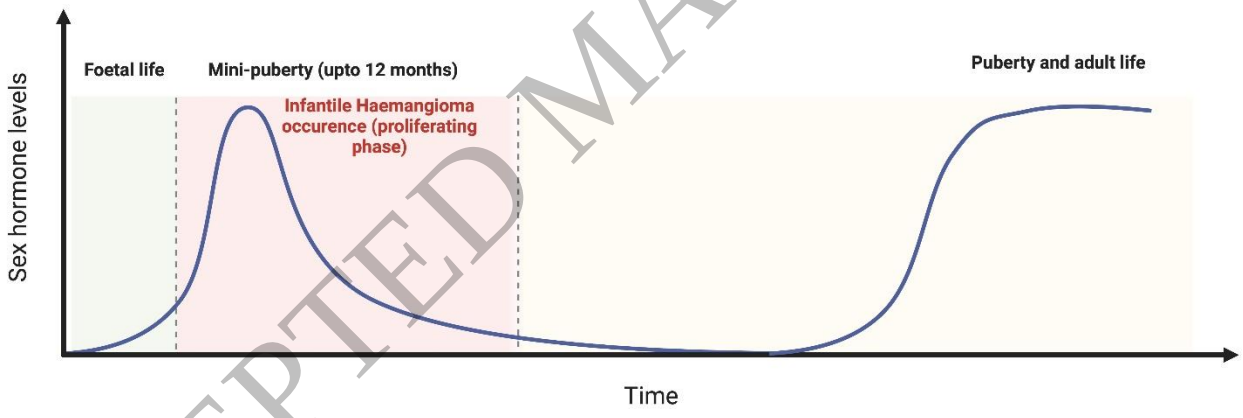

Figure 4
165x214 mm (DPI)