Br J Dermatol 2024; **00**:1–9
https://doi.org/10.1093/bjd/ljae241
Advance access publication date: 7 June 2024

BJD
British Journal of Dermatology
Review Article

Recent advances in understanding the molecular basis of infantile haemangioma development

Raka Mitra,[1,2] Helen L Fitzsimons,[2] Tracy Hale,[2] Swee T Tan,[1,4,5] Clint Gray[1,3] and Madeleine PJ White[1]

[1]Gillies McIndoe Research Institute, Newtown, Wellington, New Zealand
[2]School of Food Technology and Natural Sciences, Massey University, Palmerston North, New Zealand
[3]Centre for Biodiscovery and School of Biological Sciences, Victoria University of Wellington, Kelburn, Wellington, New Zealand
[4]Wellington Regional Plastic, Maxillofacial and Burns Unit, Hutt Hospital, Lower Hutt, New Zealand
[5]Department of Surgery, The Royal Melbourne Hospital, The University of Melbourne, Melbourne, VIC, Australia
Correspondence: Madeleine P.J. White. Email: maddie.white@gmri.org.nz

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 the underlying biological processes, with some of the key IH-related pathways identified, including vascular endothelial growth factor, the renin–angiotensin–aldosterone system, hypoxia-inducible factor 1α, Notch, platelet-derived growth factor, phosphoinositide 3-kinase/Akt/mammalian target of rapamycin, Janus kinase/signal transducers and activators of transcription, fibroblast growth factor, peroxisome proliferator-activated receptor-γ and insulin-like growth factor. 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, identifying differentially expressed genes, targeted proteins and key signalling pathways. This knowledge will increase the broader understanding of vascular development, tissue remodelling and angiogenesis.

Lay summary

Infantile haemangioma is a common vascular tumour (or birthmark) that affects babies. Less is known about the pathways involved in their development and progression.

We look at the key proteins involved in the development of these tumours. These proteins help new blood vessels grow (called 'angiogenesis') when a tumour is just starting to appear. The area around the tumour becomes inflamed, and the immune system releases molecules called 'cytokines' that help new blood vessels grow and to increase the number of cells (called 'proliferation'). Within a year, the tumours shrink and are gradually replaced by fatty tissue (called 'spontaneous involution'). This phase is influenced by other proteins called 'IGF' and 'PPAR-γ', which encourage cell death and tissue remodelling. Hormone receptors and the area outside cells called the 'extracellular matrix' could be the key to understanding this change. Even though many protein signalling pathways are involved in the two phases, we still do not know exactly how the switch between them happens.

This review explains the molecular mechanisms behind infantile haemangiomas, focusing on how the protein signalling pathways work together. Our research offers valuable insights for both researchers and clinicians in this field.

Overview of infantile haemangioma 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, White and premature infants.[3,5] Preterm babies have a 40% increased risk, with one in four developing IH.[6] Maternal factors such as advanced age, multiple pregnancies, maternal complications

Accepted: 2 June 2024

(e.g. pre-eclampsia and amniocentesis), a history of infertility treatments and conception via *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% of cases and the occurrence of >5 lesions increases the risk of liver involvement (50% of 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 are 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 (e.g. ulceration with pain and bleeding), tissue distortion and functional problems (e.g. 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 or 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 have been 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] The complications of steroid administration are well known and include insomnia, hypertension, adrenal suppression, growth retardation, gastrointestinal reflux and immune suppression.[3] Repeated intralesional steroid injections, usually performed under general anaesthesia, have been used for localized IHs and pulsed-dye laser treatment has been used for superficial ulcerated lesions.[12] Interferon-α has been used for cases of problematic proliferating IHs refractory to systemic steroids. However, in view of neurotoxicity such as spastic diplegia, this treatment has been abandoned in favour of the chemotherapeutic drug vincristine, which is associated with toxicity such as neutropenia.[7,12,13]

The nonselective beta blocker propranolol has become the mainstay of treatment for problematic proliferating IH since the serendipitous discovery in 2008 that it causes accelerated involution of IH.[14] Other beta blockers, including the β1-selective blocker atenolol, have been used to treat IH.[15] Although an effective treatment in 85–90% of cases,[16] few long-term studies have investigated the effects of beta blockers on infant development. A 2021 study by Tan *et al.* reported significant adverse side-effects of beta blockers in babies, including hypertension, hypoglycaemia, sleep disturbances and bradycardia.[17] This resulted in some patients stopping treatment early, which is not optimal as in rare cases cessation has been associated with treatment resistance or rebound of tumour growth.[7] Subtherapeutic dosages have sometimes been recommended as they are associated with low side-effect rates.[18] Another study by Hali *et al.* in 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 beta blockers such as timolol are indicated for small, superficial IH, but there have been few studies with sufficient patient numbers and limited efficacy has been seen with topical treatment.[20] Although the indications for oral beta blockers for problematic proliferating IHs have broadened from those for systemic high-dose steroids, the majority of IHs are not actively treated because of 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 systemic beta blocker administration during infancy on development and growth.

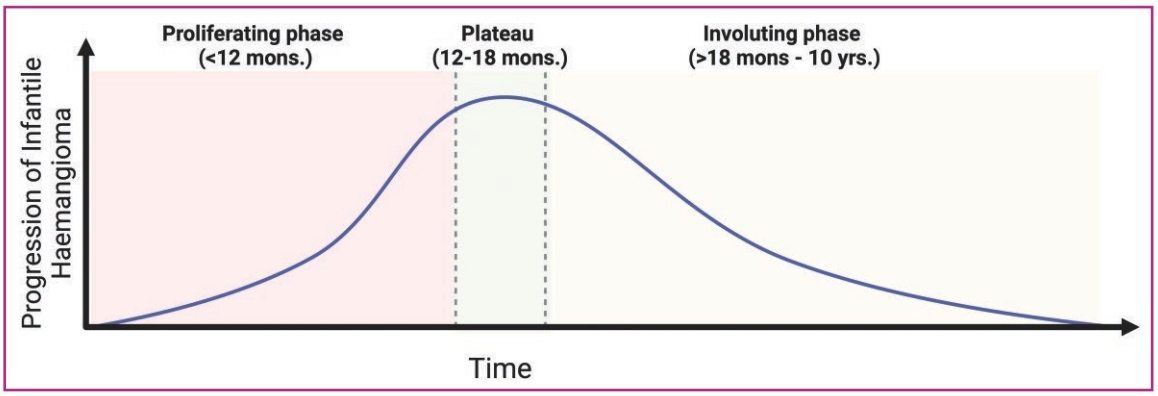

Figure 1 Overview of the phases (proliferating, plateau and involuting) in the progression of infantile haemangioma (IH) along the course of time. IH starts with the proliferating phase in the first 12 months of life; it then plateaus or goes through a quiescent phase for 12–18 months, followed by the involuting phase, which lasts for up to 10 years. Created with BioRender.com.

Molecular mechanisms of proliferating infantile haemangioma

The cells that make up 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 and 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 vascular endothelial growth factor (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 has also been demonstrated due to similarities in HemEC and placental cellular markers, such as glucose transporter 1 (GLUT1), the diagnostic marker of IH.[1,25–27] GLUT1 is a unique maker for IH, shared with placenta, and it differentiates this tumour from other vascular anomalies.[28] C19MC is the largest microRNA (miRNA) cluster in humans and consists of 46 miRNAs. Compared with other vascular anomalies, this cluster is unique to IH and circulates in the serum of patients with IH. The clusters are released by GLUT1$^+$ endothelial cells and may therefore be a potential biomarker for this tumour.[29–31] HemSCs, which constitute approximately 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 the IH microenvironment.[22,33] These cells have been associated with inflammation and regression of IH.[10] Alterations 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; these are summarized in Table 1.

Proangiogenic factors

Vasculogenesis and angiogenesis are key processes in the proliferating phase of IH, with vasculogenesis involving the 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 proangiogenic factors such as VEGF receptor (VEGFR), angiopoietin II (ANGPT2) and hypoxia inducible factor (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] VEGFRs regulate angiogenesis, promoting and maintaining blood vessel growth. Two receptors of the VEGF family are expressed in HemECs: VEGFR-1 and VEGFR-2. VEGFR-1 has higher binding affinity for its ligand than VEGFR-2 but weaker kinase

Table 1 Summary of dysregulated signalling pathways in infantile haemangioma (IH)

Signalling pathway	Components	Interactions	References
VEGF	VEGFR1, VEGFR2	VEGFR1 suppresses angiogenesis in HemECs and suppresses differentiation of HemSCs to HemECs; VEGFR2 initiates immature vessel development	34,38
HIF	HIF-1α	HIF-1α overexpression in HemECs leads to increased VEGF and GLUT1	7,16,34,37
RAAS	ANGPT2, PRR, ACE, ATII, ATIIR2	Renin promotes HemEC proliferation, ANGPT2 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 and pSTAT5 expressed in HemECs and cells within the interstitium in proliferating IH	47
PDGF	PDGFR-β	PDGFR-β inhibits differentiation of HemSCs to adipocytes; Hem-pericytes express PDGFR-β (role unknown)	32,34,48
Notch	Notch-1, Notch-3, Notch-4, HES1, HEY1, HEY2, HEYL, Jagged-1	Notch-3, HES1, HEY1 and HEYL are overexpressed in HemSCs, while Notch-1, Notch-4, Jagged-1 and HEY2 (although not uniformly expressed) levels are high in HemECs; Jagged-1 differentiates mural cells via Notch-3 activation in HemSCs during involution; DLL4 and Notch-1 activates stalk-cell conformation in HemEC	34,51,52
PPAR-γ	PPAR-γ2, CEBPα, CEBPβ, LPL, perilipin A	Adipogenesis in HemSCs, HemEC apoptosis, suppress angiogenesis	53,54
IGF	IGF-1 IGF-2	Adipogenesis in HemSCs via PI3K pathway	55,57,58

ACE, angiotensin-converting enzyme; ANGPT2, angiopoietin II; ATII, angiotensin II; ATIIR2, angiotensin II receptor 2; CEBP, CCAAT/enhancer binding protein; DLL, delta-like; ECM, extracellular matrix; ERK, extracellular regulated kinase; FGF, fibroblast growth factor; GLUT1, glucose transporter 1; HemEC, haemangioma-derived endothelial cell; HemSC, haemangioma-derived stem cell; HES1, hairy and enhancer of split 1; HEY, HES-related protein; HEYL, HES related with YRPW motif-like protein; HIF, hypoxia inducible factor; IGF, insulin-like growth factor; JAK, Janus kinase; mTOR, mammalian target of rapamycin; LPL, lipoprotein lipase; PDGF, platelet-derived growth factor; PDGFR-β, PDGR receptor β; PI3K, phosphoinositide 3-kinase; PPAR, peroxisome proliferator-activated receptor; PRR, prorenin receptor; pSTAT, phosphorylated STAT; RAAS, renin–angiotensin–aldosterone system; STAT, signal transducers and activators of transcription; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

activity. Endothelial cells expressing high levels of VEGFR-2 show superior growth.[34] Decreased VEGFR-1 expression is seen in proliferating HemECs, owing to its role in suppressing angiogenesis. However, VEGFR-1 expression is increased in HemSCs, leading to their differentiation through the activation of VEGFR-1 by VEGF-A. HemECs are then stimulated by VEGFR-2 to undergo immature vessel development (Figure 2). Consistent Bcl-2 expression prevents apoptosis of HemEC through the constitutively active autocrine VEGF-A/VEGFR-2 loop, further promoting growth during the IH proliferative phase. Other downstream pathways activated by VEGF include Ras/Raf/mitogen-activated protein kinase (MEK)/extracellular regulated kinase (ERK), phosphoinositide 3-kinase (PI3K)/Akt and protein kinase C, 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, fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), among others.[16] The transcription factor HIF-1α is expressed at a higher level by HemECs than normal endothelial cells, leading to upregulated VEGF and GLUT1 expression. Propranolol suppresses the survival of HemECs by targeting this HIF-1α/VEGF pathway.[4,34]

The renin–angiotensin–aldosterone 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 in IH and is localized to nonendothelial and endothelial IH cell populations.[40] Renin – a glycoproteolytic enzyme – catalyses angiotensinogen into angiotensin I. 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, ATIIR2, angiotensin-converting enzyme (ACE) 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, ACE and angiotensin II decrease in

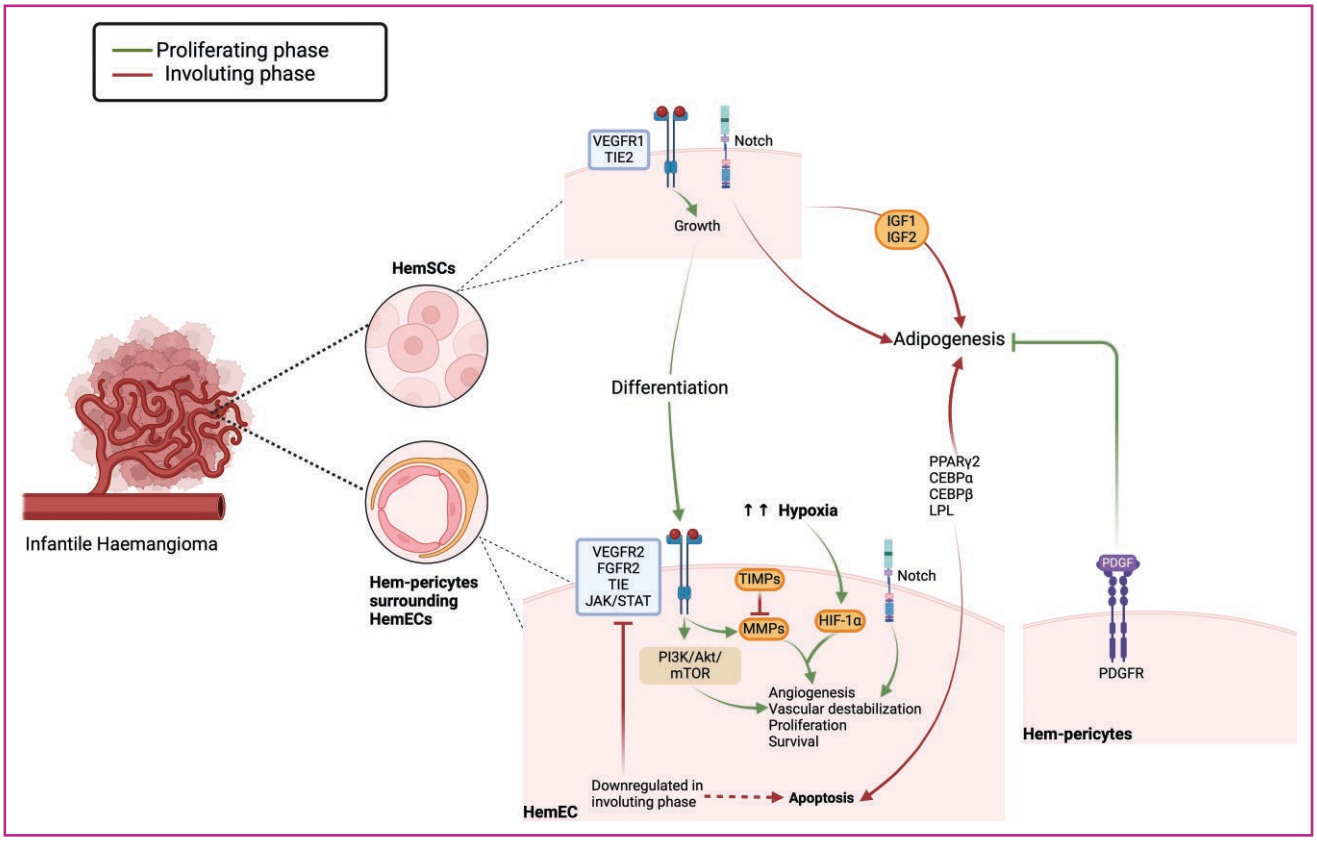

Figure 2 Molecular mechanisms involved in the pathogenesis of infantile haemangioma (IH). Haemangioma stem cells (HemSCs), endothelial cells (HemECs) and pericytes (Hem-pericytes) play different roles in the proliferating and involuting phases. HemECs actively divide and form blood vessels due to upregulated vascular endothelial growth factor (VEGF), Notch, fibroblast growth factor 2 (FGF2), TIE/renin–angiotensin–aldosterone system (RAAS), Janus kinase (JAK)/signal transducers and activators of transcription (STAT), and phosphoinositide 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) signalling pathways. HemSCs stimulate HemECs via the VEGF and TIE2 pathway and Hem-pericytes prevent adipogenesis [platelet-derived growth factor (PDGF)-β pathway]. In the involuting phase, adipogenic differentiation of HemSCs [peroxisome proliferator-activated receptor (PPAR-γ), Notch pathway] and apoptosis of HemECs [downregulation of angiogenic factors like matrix metalloproteinases (MMPs) by tissue inhibitor of metalloproteinases (TIMPs) and other proliferative signalling pathways] occurs. CEBP, CCAAT/enhancer binding protein; FGFR2, FGF receptor 2; HIF-1α, hypoxia inducible factor 1α; IGF, insulin-like growth factor; LPL, lipoprotein lipase; PDGFR, PDGF receptor; VEGFR1, VEGF receptor 1. Created with BioRender.com.

patients treated with 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 (Table 1).[40,44] One of the mechanisms by which beta blockers may induce accelerated involution of IH is by targeting the RAAS pathway, which is upregulated during the proliferating phase of IH.[18]

Signalling pathways that promote cellular proliferation in infantile haemangioma

Aberrant cell proliferation in IH is induced by FGF receptor (FGFR), PI3K/Akt/mammalian target of rapamycin (mTOR), Janus kinase (JAK)/signal transducers and activators of transcription (STAT) and PDGF signalling pathways. Overexpression of FGF2 [also known as basic FGF (bFGF)] occurs during the proliferative phase of IH and is downregulated during the involuting and involuted phases. Furthermore, FGF2 is a specific angiogenic factor that stimulates endothelial cell proliferation and promotes fibroblast migration, as well as the generation of collagen, fibronectin and integrin (Table 1). FGF2 binds to the FGFR1 and inhibits ERK1/2 phosphorylation and PI3K stimulation. This subsequently initiates downstream signalling of mTOR.[23,39] HemECs have an active P13 K/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 and differentiation of HemSCs, although this treatment in infants is associated with side-effects.[34] miR-126 and miR-210 are proangiogenic and stimulate HemEC proliferation by bFGF and VEGF signalling.[45,46]

Phosphorylated and activated forms of STAT1, STAT3 and STAT5 of the JAK/STAT signalling pathway have 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. ATIIR2 induces STAT3, influencing IH growth via the RAAS pathway.[47] The PDGF family comprises ligands 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.[48] HemSCs also give rise to Hem-pericytes, which surround HemECs, controlling differentiation and proliferation.[32] Moreover, the endothelium is an important source of 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 (MMPs) are a family of protein and peptide hydrolases 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 break down 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 collagens IV, V, VII, IX and X, all of which promote EC migration and the formation of a capillary network.[38,49] 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

Antiangiogenic and proangiogenic factors

Antiangiogenic pathways, including tissue inhibitor of metalloproteinase (TIMP) and Notch, are upregulated in involuting IH.[34,49] TIMPs suppress the activation of pro-MMPs thereby inhibiting ECM degradation and angiogenesis (Figure 2). They have multiple functions, including the 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.[49]

The Notch signalling pathway is a vital cascade in IH. It encompasses both pro- and antiangiogenic factors, playing a pivotal role in balancing the angiogenic switch, which can influence the progression and involution of these tumours. Notch receptors (Notch 1–4) interact with ligands [delta-like (DLL)1, DLL3, DLL4; Jagged-1, Jagged-2] that cleave transmembrane Notch receptor, resulting in the migration of the Notch intracellular domain 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 (HES related with YRPW motif-like protein) are overexpressed in HemSCs, while Notch 1, Notch 4, Jagged-1 and HEY2 levels (though not uniformly expressed) are high in HemECs (Table 1).[34,50] Interestingly, some studies have reported that Jagged-1 expression in HemECs results in mural cell differentiation via 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.[51]

Laser capture microdissection and genome-wide transcriptional profiling have shown high *NOTCH4* and *JAG1* expression in the proliferative phase of IH vs. placenta.[52] An imbalance in *JAG1* and *DLL4* expression is more prevalent in HemECs than in HemSCs. *JAG2* mRNA and Jagged-2 protein are highly expressed in proliferating IH vs. involuting lesions, with no *DLL3* expression.[51] Interestingly, loss of DLL3 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. However, DLL1 interacts with Jagged-1 to antagonize vascular development. In several studies, the VEGF and Notch pathways have been found to interact with each other, although with contradictory findings on their regulation in IH. VEGF increases DLL4 expression, although VEGFR-2 expression decreases Notch and VEGFR-1 activity, which drives the proliferation of ECs.[34,51] More research is needed to elucidate the subtle changes in gene expression and molecular adaptations within these pathways before and after phase transition, to further improve 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,[23,53] resulting in the regression of blood vessels and tumour shrinkage, although the trigger has not yet been clearly established. The nuclear receptor peroxisome proliferator-activated receptor (PPAR)-γ promotes a proadipogenic and anti-inflammatory response during involution and can interact with oestrogen to regulate adipogenesis.[32] Perivascular mesenchymal stem cells, also known as pericytes, regulate adipogenesis in IH by the PPAR-γ pathway and differentiate into adipocytes during the involuting phase.[54] During the involuting phase, there is an increase in the expression of PPAR- γ2, lipoprotein lipase, CCAAT/enhancer binding protein (CEBP)α and perilipin A that is associated with the differentiation of IH mesenchymal stem cells to adipocytes (Table 1).[53] This suggests the PPAR-γ pathway plays a vital role in suppressing angiogenesis and increasing apoptosis of HemECs in the involuting phase (Figure 2).[53] Cyclooxygenase-2 inhibition has also been hypothesized to be correlated with the PPAR-γ/CEBP signalling pathway to promote involution.[55] In another study, expression levels of CEBPα, CEBPβ, PPAR-γ and adiponectin were found to be increased in HemSC during adipogenesis when treated with *N*-[*N*-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT), an inhibitor of the Notch pathway.[56]

The insulin-like 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.[57] IGF-2 also induces HemSC adipogenic differentiation via the same mechanism.[55,58] 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 the development of treatments targeting the factors that trigger involution.

Hormonal influence on infantile haemangioma

IH is more common in females, with research showing a female-to-male ratio of up to 3 : 1.[3,5] Sex-based differences in the 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 may explain, in part, the increased prevalence in females. Growing evidence suggests a connection between oestrogen and IH, with one study reporting increased serum oestradiol levels in children with IH vs. 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 2021 study by Johnson *et al.* reported 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 oestrogen: 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 (ERα and ERβ) and cell membrane receptors [G protein-coupled receptor 30 (GPR30) and ER-X]. The nuclear receptors are ligand-regulated transcription factors that control the expression of genes required for the development of the reproductive system. E2 and ERα are highly expressed in mural cells in IH but not in endothelial cells. Mural cell precursors are attracted to the tumour by E2 and can migrate into the IH tissue and mature; once activated, they secrete proangiogenic (FGF2) and antiangiogenic factors [interferon (IFN)-α, IFN-β, IFN-γ, transforming growth factor-β] in the tumour microenvironment, which are taken up by the adjacent cells, including fibroblasts, plasma cells and macrophages, and may be involved in the involution of IH (Figure 3).[60] ERα and ERβ are present on adipocytes and their expression can alter during differentiation, indicating that 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] *ESR1* and *PGR* mRNA and protein (ERα and PR) levels also increase during involution of IH vs. the proliferative phase, although *ESR2* mRNA does not relate to protein expression (ERβ) owing to post-transcriptional modifications.[62]

Surprisingly, oestrogen levels are higher in newborn babies with IH; this is associated with higher VEGF expression. Limited studies have shown that tamoxifen – a selective ER modulator commonly used in the treatment of breast cancer – lowers VEGF expression in an ER-dependent manner.[65] PR receptors are the most highly expressed of the hormone receptors in IH, although research on the significance of this expression in IH is lacking, raising the possibility that hormone-based treatments during pregnancy will increase the risk of IH.[62] The levels of circulating 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 a gradual decline. This correlates 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 than in the involuting phase. Clinically, the levels of FSH are higher in females than in males, and are 40% higher in low-birthweight and preterm newborn babies,

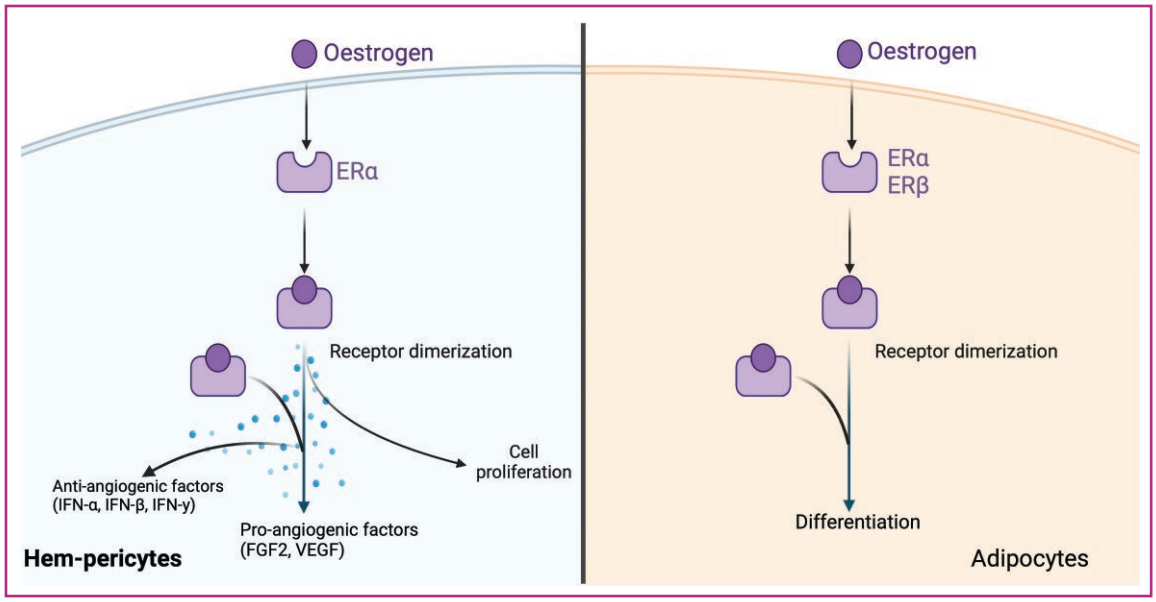

Figure 3 Oestrogen hormone regulation in infantile haemangioma during the proliferating and involuting phases in pericytes and adipocytes, respectively. Both proangiogenic [fibroblast growth factor 2 (FGF2), vascular endothelial growth factor (VEGF)] and antiangiogenic [interferon (IFN)-α, IFN-β, IFN-γ] factors are released in pericytes, whereas oestrogen activation leads to differentiation of adipocytes during regression. ERα, oestrogen receptor α; ERβ, oestrogen receptor β. Created with BioRender.com.

correlating to the incidence of IH with increased FSH levels within the first 12 months of life (Figure 4).[67,68] FSH promotes HemSC proliferation and cell cycle progression (G1/S phase transition),[69] while another study showed that it inhibits apoptosis by stimulating protein kinase B (Akt),[70] upregulating VEGFR2 and antiapoptotic 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 the progression and involution of IH and to 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 the 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 the programmed biologic 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 that promote cell survival. Several genes that are significantly upregulated in IH are implicated in the VEGF, FGF, PI3K/Akt/mTOR, RAAS, HIF-1α, Notch and PDGF pathways.[23,34]

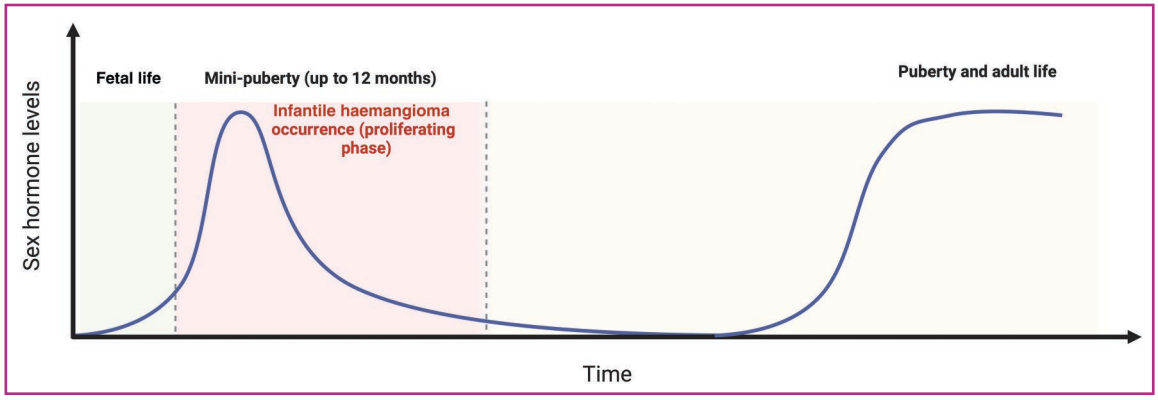

Figure 4 Changes in sex hormone levels in the first year of life in infants correlated to the occurrence of infantile haemangioma (IH; proliferating phase). IH occurrence has been found to be associated with the increase in hormonal levels in the first 12 months of life, also known as 'mini-puberty'.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

Decreased angiogenesis with subsequent adipogenesis is important for progression to the involuting phase. Stopping or reducing IH growth by targeting HemSCs and promoting regression has been a challenge that researchers must address. By unravelling the intricacies involved in these gene mutations, we will be able to improve the management of IH, reduce side-effects and improve the quality of life of affected individuals. There is scope for advancing haemangioma research and treatment in terms of topical vs. oral drugs, genomic and proteomic profiling, and investigating the role of diverse cell types involved in the progression of IH. Therefore, stage and sex may be crucial factors in the development of future treatments, but this requires further research to understand fully 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 by addressing these problems can a more seamless translation of IH research into new and effective strategies to treat infants with IH be achieved.

Funding sources

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Conflicts of interest

The authors declare no conflicts of interest.

Data availability

No new data were generated.

Ethics statement

Not applicable.

Patient consent

Not applicable.

References

1 Eisenstein KA. Infantile hemangiomas: a review and future opportunities. *Mo Med* 2023; **120**:49–52.
2 Nguyen HL, Boon LM, Vikkula M. Genetics of vascular anomalies. *Semin Pediatr Surg* 2020; **29**:150967.
3 Lee KC, Bercovitch L. Update on infantile hemangiomas. *Semin Perinatol* 2013; **37**:49–58.
4 Chen ZY, Wang QN, Zhu YH *et al.* Progress in the treatment of infantile hemangioma. *Ann Transl Med* 2019; **7**:692.
5 Lin Q, Cai B, Shan X *et al.* Global research trends of infantile hemangioma: a bibliometric and visualization analysis from 2000 to 2022. *Heliyon* 2023; **9**:e21300.
6 Garzon MC, Drolet BA, Baselga E *et al.* Comparison of infantile hemangiomas in preterm and term infants: a prospective study. *Arch Dermatol* 2008; **144**:1231–2.
7 Xu W, Zhao H. Management of infantile hemangiomas: recent advances. *Front Oncol* 2022; **12**:1064048.
8 Torres E, Rosa J, Leaute-Labreze C, Soares-de-Almeida L. Multifocal infantile haemangioma: a diagnostic challenge. *BMJ Case Rep* 2016; **2016**:bcr2016214827.
9 Nazemian S, Sharif S, Childers ELB. Infantile hemangioma: a common lesion in a vulnerable population. *Int J Environ Res Public Health* 2023; **20**:5585.
10 Greenberger S, Bischoff J. Infantile hemangioma – mechanism(s) of drug action on a vascular tumor. *Cold Spring Harb Perspect Med* 2011; **1**(1):a006460–a006460.
11 Cheng CE, Friedlander SF. Infantile hemangiomas, complications and treatments. *Semin Cutan Med Surg* 2016; **35**:108–16.
12 Tan BH, Leadbitter PH, Aburn NH, Tan ST. Steroid therapy for problematic proliferating haemangioma. *N Z Med J* 2011; **124**:57–65.
13 Sebaratnam DF, Rodríguez Bandera AL, Wong LCF, Wargon O. Infantile hemangioma. Part 2: Management. *J Am Acad Dermatol* 2021; **85**:1395–404.
14 Léauté-Labrèze C, Dumas de la Roque E, Hubiche T *et al.* Propranolol for severe hemangiomas of infancy. *N Engl J Med* 2008; **358**:2649–51.
15 Ji Y, Chen S, Yang K *et al.* Efficacy and safety of propranolol vs atenolol in infants with problematic infantile hemangiomas: a randomized clinical trial. *JAMA Otolaryngol Neck Surg* 2021; **147**:599–607.
16 Kowalska M, Dębek W, Matuszczak E. Infantile hemangiomas: an update on pathogenesis and treatment. *J Clin Med* 2021; **10**:4631.
17 Tan X, Guo S, Wang C. Propranolol in the treatment of infantile hemangiomas. *Clin Cosmet Investig Dermatol* 2021; **14**:1155–63.
18 Koh SP, Leadbitter P, Smithers F, Tan ST. β-blocker therapy for infantile hemangioma. *Expert Rev Clin Pharmacol* 2020; **13**:899–915.
19 Hali F, Moubine I, Berrami H *et al.* Predictors of poor response to oral propranolol in infantile hemangiomas. *Arch Pediatr* 2023; **30**:455–7.
20 Anwar F, Mahmood E, Sharif S *et al.* Topical application of 0.5% timolol maleate hydrogel for the treatment of superficial infantile hemangiomas. *J Drugs Dermatol* 2023; **22**:594–8.
21 Tan EMS, Blackwell MG, Dunne JC *et al.* Neuropeptide Y receptor 1 is expressed by B and T lymphocytes and mast cells in infantile haemangiomas. *Acta Paediatr* 2017; **106**:292–7.
22 Tan EMS, Chudakova DA, Davis PF *et al.* Characterisation of subpopulations of myeloid cells in infantile haemangioma. *J Clin Pathol* 2015; **68**:571–4.
23 Sun Y, Qiu F, Hu C *et al.* Hemangioma endothelial cells and hemangioma stem cells in infantile hemangioma. *Ann Plast Surg* 2022; **88**:244–9.
24 Boscolo E, Mulliken JB, Bischoff J. Pericytes from infantile hemangioma display pro-angiogenic properties and dysregulated angiopoietin-1. *Arterioscler Thromb Vasc Biol* 2013; **33**:501–9.
25 Itinteang T, Tan ST, Brasch HD *et al.* Primitive erythropoiesis in infantile haemangioma. *Br J Dermatol* 2011; **164**:1097–100.
26 Itinteang T, Tan ST, Guthrie S *et al.* A placental chorionic villous mesenchymal core cellular origin for infantile haemangioma. *J Clin Pathol* 2011; **64**:870–4.
27 Rodríguez Bandera AI, Sebaratnam DF, Wargon O, Wong LCF. Infantile hemangioma. Part 1: Epidemiology, pathogenesis, clinical presentation and assessment. *J Am Acad Dermatol* 2021; **85**:1379–92.
28 North PE, Waner M, Mizeracki A, Mihm MC. GLUT1: a newly discovered immunohistochemical marker for juvenile hemangiomas. *Hum Pathol* 2000; **31**:11–22.
29 Strub GM, Kirsh AL, Whipple ME *et al.* Endothelial and circulating C19MC microRNAs are biomarkers of infantile hemangioma. *JCI Insight* 2016; **1**:e88856.
30 Fu C, Lv R, Xu G *et al.* Circular RNA profile of infantile hemangioma by microarray analysis. *PLOS ONE* 2017; **12**:e0187581.
31 Fu C, Yang K, Zou Y, Huo R. Identification of key microRNAs and genes in infantile hemangiomas. *Front Genet* 2022; **13**:766561.

32 Harbi S, Wang R, Gregory M *et al.* Infantile hemangioma originates from a dysregulated but not fully transformed multipotent stem cell. *Sci Rep* 2016; **6**:35811.
33 Itinteang T, Tan ST, Jia J *et al.* Mast cells in infantile haemangioma possess a primitive myeloid phenotype. *J Clin Pathol* 2013; **66**:597–600.
34 Ji Y, Chen S, Li K *et al.* Signaling pathways in the development of infantile hemangioma. *J Hematol Oncol* 2014; **7**:13.
35 Boye E, Olsen BR. Signaling mechanisms in infantile hemangioma. *Curr Opin Hematol* 2009; **16**:202.
36 Heredea RE, Melnic E, Cirligeriu LE *et al.* VEGF pathway gene expression profile of proliferating versus involuting infantile hemangiomas: preliminary evidence and review of the literature. *Child Basel Switz* 2022; **9**:908.
37 de Jong S, Itinteang T, Withers AHJ *et al.* Does hypoxia play a role in infantile hemangioma? *Arch Dermatol Res* 2016; **308**:219–27.
38 Yin RR, Hao D, Chen P. Expression and correlation of MMP-9, VEGF, and p16 in infantile hemangioma. *Eur Rev Med Pharmacol Sci* 2018; **22**:4806–11.
39 El-Raggal NM, El-Farrash RA, Saad AA *et al.* Circulating levels of vascular endothelial growth factor and basic fibroblastic growth factor in infantile hemangioma versus vascular malformations. *Clin Appl Thromb* 2018; **24**:663–8.
40 van Schaijik B, Tan ST, Marsh RW, Itinteang T. Expression of (pro) renin receptor and its effect on endothelial cell proliferation in infantile hemangioma. *Pediatr Res* 2019; **86**:202–7.
41 Itinteang T, Brasch HD, Tan ST, Day DJ. Expression of components of the renin-angiotensin system in proliferating infantile haemangioma may account for the propranolol-induced accelerated involution. *J Plast Reconstr Aesthetic Surg* 2011; **64**:759–65.
42 Itinteang T, Marsh R, Davis PF, Tan ST. Angiotensin II causes cellular proliferation in infantile haemangioma via angiotensin II receptor 2 activation. *J Clin Pathol* 2015; **68**:346–50.
43 Sulzberger L, Baillie R, Itinteang T *et al.* Serum levels of renin, angiotensin-converting enzyme and angiotensin II in patients treated by surgical excision, propranolol and captopril for problematic proliferating infantile haemangioma. *J Plast Reconstr Aesthetic Surg* 2016; **69**:381–6.
44 Dornhoffer JR, Wei T, Zhang H *et al.* The expression of renin–angiotensin–aldosterone axis components in infantile hemangioma tissue and the impact of propranolol treatment. *Pediatr Res* 2017; **82**:155–63.
45 Cao Z, Guo YQ, Tang SJ *et al.* Transfection of adenovirus-mediated mircoRNA-126 gene into infant hemangioma endothelial cells in vitro. *Int J Clin Exp Pathol* 2018; **11**:1811–17.
46 Bertoni N, Pereira LMS, Severino FE *et al.* Integrative meta-analysis identifies microRNA-regulated networks in infantile hemangioma. *BMC Med Genet* 2016; **17**:4.
47 Sulzberger L, Tan EMS, Davis PF *et al.* Phosphorylated forms of STAT1, STAT3 and STAT5 are expressed in proliferating but not involuted infantile hemangioma. *Front Surg* 2018; **5**:31.
48 Roach EE, Chakrabarti R, Park NI *et al.* Intrinsic regulation of hemangioma involution by platelet-derived growth factor. *Cell Death Dis* 2012; **3**:e328.
49 Zhong S, Yang G, Xia C *et al.* Expression of matrix metalloproteinase and its tissue inhibitor in haemangioma. *J Huazhong Univ Sci Technolog Med Sci* 2009; **29**:614–19.
50 Wu JK, Adepoju O, De Silva D *et al.* A switch in Notch gene expression parallels stem cell to endothelial transition in infantile hemangioma. *Angiogenesis* 2010; **13**:15.
51 Zhang H, Wei T, Johnson A *et al.* NOTCH pathway activation in infantile hemangiomas. *J Vasc Surg Venous Lymphat Disord* 2021; **9**:489–96.
52 Calicchio ML, Collins T, Kozakewich HP. Identification of signaling systems in proliferating and involuting phase infantile hemangiomas by genome-wide transcriptional profiling. *Am J Pathol* 2009; **174**:1638–49.
53 Yuan SM, Guo Y, Wang Q *et al.* Over-expression of PPAR-γ2 gene enhances the adipogenic differentiation of hemangioma-derived mesenchymal stem cells in vitro and in vivo. *Oncotarget* 2017; **8**:115817–28.
54 Wnęk A, Andrzejewska E, Kobos J *et al.* Molecular and immunohistochemical expression of apoptotic proteins Bax, Bcl-2 and Caspase 3 in infantile hemangioma tissues as an effect of propranolol treatment. *Immunol Lett* 2017; **185**:27–31.
55 Wang F, Li H, Lou Y *et al.* Insulin–like growth factor I promotes adipogenesis in hemangioma stem cells from infantile hemangiomas. *Mol Med Rep* 2019; **19**:2825–30.
56 Xu X, Wu Y, Li H *et al.* Notch pathway inhibitor DAPT accelerates in vitro proliferation and adipogenesis in infantile hemangioma stem cells. *Oncol Lett* 2021; **22**:854.
57 Xu M, Ouyang T, Lv K, Ma X. Integrated WGCNA and PPI network to screen hub genes signatures for infantile hemangioma. *Front Genet* 2021; **11**:614195.
58 Li J, Li Q, Chen L *et al.* Expression profile of circular RNAs in infantile hemangioma detected by RNA-Seq. *Medicine (Baltimore)* 2018; **97**:e10882.
59 Mulliken JB, Burrows PE, Fishman SJ (eds). *Mulliken and Young's Vascular Anomalies: Hemangiomas and Malformations*. Oxford University Press, 2013.
60 Hou F, Dai Y, Fan CY *et al.* Estrogen is involved in hemangioma regression associated with mast cells. *Orphanet J Rare Dis* 2018; **13**:181.
61 Sun ZY, Yang L, Yi CG *et al.* Possibilities and potential roles of estrogen in the pathogenesis of proliferation hemangiomas formation. *Med Hypotheses* 2008; **71**:286–92.
62 Johnson A, Zhang H, Gonzalez SR *et al.* Presence of estrogen and progesterone receptors in proliferating and involuting infantile hemangiomas. *J Plast Reconstr Aesthet Surg* 2021; **74**:3061–5.
63 Paterni I, Granchi C, Katzenellenbogen JA, Minutolo F. Estrogen receptors alpha (ERα) and beta (ERβ): subtype-selective ligands and clinical potential. *Steroids* 2014; **90**:13–29.
64 Pedersen SB, Bruun JM, Hube F *et al.* Demonstration of estrogen receptor subtypes α and β in human adipose tissue: influences of adipose cell differentiation and fat depot localization. *Mol Cell Endocrinol* 2001; **182**:27–37.
65 Xiao X, Liu J, Sheng M. Synergistic effect of estrogen and VEGF on the proliferation of hemangioma vascular endothelial cells. *J Pediatr Surg* 2004; **39**:1107–10.
66 Haldar S, Agrawal H, Saha S *et al.* Overview of follicle stimulating hormone and its receptors in reproduction and in stem cells and cancer stem cells. *Int J Biol Sci* 2022; **18**:675–92.
67 Maclellan RA, Konczyk DJ, Goss JA, Greene AK. Analysis of follicle-stimulating hormone receptor in infantile hemangioma. *Ann Plast Surg* 2018; **80**:S211.
68 Wildgruber M, Sadick M, Müller-Wille R, Wohlgemuth WA. Vascular tumors in infants and adolescents. *Insights Imaging* 2019; **10**:30.
69 Hong Z, Kuang J, Guo Y *et al.* Effects of follicle-stimulating hormone on the proliferation and apoptosis of infantile hemangioma stem cells. *Biochem Biophys Rep* 2023; **35**:101551.
70 Munabi NCO, England RW, Edwards AK *et al.* Propranolol targets hemangioma stem cells via cAMP and mitogen-activated protein kinase regulation. *Stem Cells Transl Med* 2016; **5**:45–55.