Angiogenesis in metabolic-vascular disease

Georg Breier*1; Triantafyllos Chavakis*2; Emilio Hirsch*3

*Division of Medical Biology, Department of Psychiatry, Faculty of Medicine and University Hospital Carl Gustav Carus, TU Dresden, Germany; 1Institute for Clinical Chemistry and Laboratory Medicine, Faculty of Medicine and University Hospital Carl Gustav Carus, TU Dresden, Germany; 3Department of Molecular Biotechnology and Health Sciences, University of Torino, Torino, Italy

Summary
Angiogenesis, literally formation of new blood vessels, is the main process through which the vascular system expands during embryonic and postnatal development. Endothelial cells, which constitute the inner lining of all blood vessels, are typically in a quiescent state in the healthy adult organism. However, in vascular and metabolic diseases, the endothelium becomes unstable and dysfunctional. The resulting tissue hypoxia may thereby induce pathological angiogenesis, which is a hallmark of disease conditions like cancer or diabetic retinopathy. However, recent evidence suggests that angiogenesis is also a major player in the context of further metabolic diseases, especially in obesity. In particular, deregulated angiogenesis is linked with adipose tissue dysfunction and insulin resistance. On the other hand, signalling pathways, such as the PI3K pathway, may regulate metabolic activities in the endothelium. Endothelial cell metabolism emerges as an important regulator of angiogenesis. This review summarises the role of angiogenesis in metabolic-vascular disease, with specific focus on the role of angiogenesis in obesity-related metabolic dysfunction and on signaling pathways, especially PI3K, linking cell metabolism to endothelial function.

Keywords
Angiogenesis, diabetes, obesity, PI3-kinase, insulin resistance, metabolism, glycolysis

Angiogenesis in metabolic-vascular disease: General aspects
In healthy tissues, blood vessel endothelial cells are quiescent and display numerous cell-cell contacts, formed by vascular endothelial (VE)-cadherin and various other cell adhesion molecules (1, 2). Angiogenic stimuli, particularly tissue hypoxia, promote the formation of vascular sprouts composed of migratory tip cells and proliferating stalk cells (3). Angiogenesis is a hallmark of various diseases, including cancer or proliferative diabetic retinopathy. Such pathological vessel growth is uncontrolled, involves compromised endothelial and vascular integrity and leads to irregular vascular networks with disturbed blood flow, and hence, disturbed tissue homeostasis (1).

Angiogenesis is orchestrated by a plenitude of growth factors and signalling pathways. The cytokine vascular endothelial growth factor (VEGF, or VEGF-A) lies in the center of the regulatory network controlling angiogenesis in both physiological and pathophysiological settings (1). VEGF triggers vascular sprouting primarily by activating the VEGF receptor-2 (VEGFR2) and downstream signalling pathways in endothelial cells. Yet, in pathological conditions, angiogenic signalling is deregulated, often as a result of hypoxia-driven VEGF upregulation. Anti-VEGF treatment, which was initially developed for antiangiogenic tumour therapy (4, 5) has become a standard therapy for diabetic or age-related retina disease (6). Further growth factors that modulate angiogenic signalling, regulate cellular interactions during sprouting and control vessel maturation and stability include the Notch-Delta system, the Angiopoietin-Tie system, Platelet-derived growth factor (PDGF) and others (1, 5, 7).

Notably, recent evidence suggests the involvement of angiogenesis in several metabolic-vascular diseases over and above diabetic vascular complications. For instance, angiogenesis has emerged as a player in obesity and insulin resistance development. In addition, new findings point to an intertwined crosstalk between endothelial function and both cellular and systemic metabolism. In particular, endothelial cell metabolism emerges as a major regulator of angiogenic functions of the endothelium (8). The present review will focus on this novel bidirectional crosstalk between angiogenesis and metabolism. On the one hand, we will review the role of angiogenesis in obesity-related metabolic disease; on the other hand, we will discuss how endothelial signalling pathways, with a special emphasis on the PI3K pathway, orchestrate the interaction between endothelial function and cell metabolism in the context of metabolic-vascular disease.
Angiogenesis in obesity

Obesity, the hallmark of which is adipose tissue (AT) expansion, is a condition that predisposes to the development of insulin resistance, type 2 diabetes and cardiometabolic disease (9, 10).

AT primarily functions to store energy in the form of lipids. However, AT is also an important modulator of systemic metabolic homeostasis, by exerting endocrine activity (11, 12). Besides the white subcutaneous and visceral AT that produces and secretes adipokines with multiple regulatory effects on energy metabolism (13, 14), recently, significant attention has been drawn at other AT forms, the brown and beige AT (15). Brown AT is capable of energy dissipation and of cold-induced heat generation mediated by the function of Uncoupling protein 1 (UCP1), also called thermogenin, which uncouples the respiratory chain (11, 14, 15). The term beige AT is used to describe a white AT depot with “brown-like” energy-dispensing properties including UCP1 expression; beige adipogenesis in white AT is induced by cold and/or catecholaminergic stimulation (14–16). Beige and brown adipogenesis are diminished in obesity (17, 18) and human BAT reciprocally correlates with body-mass index (19).

Experimental evidence accumulated over the last two decades point to a major role of inflammation in the AT for the pathogenesis of obesity-related metabolic dysfunction (9, 10, 20, 21). Several immune cell populations are located in the AT and their composition may change in the course of obesity (9, 21–23). For instance, cells of type 2 immunity, such as alternatively activated (M2-like) macrophages, eosinophils, and type 2 innate lymphoid cells (ILC2) are important regulators of lean AT homeostasis, including the beige adipogenic capacity of the white AT, as well as of glucose homeostasis, thereby counteracting obesity (23–26).

In contrast, accumulation of inflammatory cells, such as classically activated (M1-like) macrophages or CDB8+ T cells, in the AT in the course of obesity contributes to insulin resistance as well as to AT metabolic dysfunction, including impaired beige adipogenesis (21, 22, 27, 28).

Interestingly, an intimate crosstalk between AT inflammation and angiogenesis exists in the context of AT dysfunction (10). Angiogenesis has been increasingly implicated in obesity and as regulator of the function of both white and brown AT (29, 30). Angiogenesis in the AT may be triggered by pro-angiogenic factors, such as VEGF (Figure 1) and is thought to support AT expansion (29, 31, 32). However, insufficient angiogenesis in the obese AT may lead to hypoxia and metabolic AT dysfunction (33, 34). Notably, oxygen partial pressure in AT of obese human subjects was reduced, as compared to AT from lean subjects; reduced oxygen partial pressure was associated with reduced vessel density and enhanced inflammation in the AT of obese subjects (35). Consistently, the angiogenic capacity of white AT in humans is decreased with obesity (31, 36). This may be due to the enhanced expression of the anti-angiogenic VEGF isoform, VEGF-A165b, in the obese AT (36). Interestingly, serum VEGF-A165b levels were significantly higher in obesity and were decreased upon bariatric surgery (36). VEGF-A165b expression in the AT of obese humans was positively regulated by Wingless-related integration site 5a (WNT5A); WNT5A thereby functioned as an inhibitor of AT angiogenesis (37).

Insufficient vascularisation of the AT has a negative impact on white, brown and beige AT. For instance, adipocyte-specific deletion of VEGF resulted in reduced vascularisation of the AT, accompanied by enhanced metabolic dysfunction and inflammation of the AT in obesity, worsened glucose tolerance, increased insulin resistance and hepatosteatosis (34). Moreover, vessel rarefaction in the brown AT resulting from obesity or adipocyte-specific deletion of VEGF promoted brown AT dysregulation and „whitening” through mitochondrial dysfunction and increased mitophagy (18).

On the contrary, transgenic mice with overexpression of VEGF in adipocytes had not only enhanced vascularisation of white and brown AT and BAT but also improved insulin sensitivity and glucose tolerance in high fat diet-induced obesity, associated with a rather anti-inflammatory switch in the AT environment. Moreover, VEGF overexpression in adipocytes resulted in increased thermogenesis and energy expenditure (38). Additionally, enhanced angiogenesis associated with increased peroxisome proliferator-activated receptor y (PPARy)-dependent expression of VEGF-A protected mice lacking the enzyme 11beta-hydroxysteroid dehydrogenase type 1, which converts cortisol to the active cortisol, from obesity (39). Recently, the VEGF-B / VEGFR1 axis was shown to prevent obesity-related metabolic dysregulation via...
modulation of VEGF / VEGFR2 signalling and AT vascularity (40). Furthermore, VEGFR2 activation in endothelial cells promotes PDGF-CC expression, which in turn induces beige adipogenesis in the white AT (Figure 1), via stimulating expression of the cardinal thermogenic factor UCP1, which hallmarks beige and brown adipocytes (41).

Additionally, insulin and insulin-like growth factor-1 stimulate angiogenesis in the AT and AT expansion; these actions are counter-acted by insulin-like growth factor-binding protein-4 (42). Angiopoietin-2 (Ang-2) is a further factor in the AT that regulates AT angiogenesis. Specifically, mice with inducible AT-specific overexpression of Ang-2 displayed elevated vessel density in the subcutaneous AT, were protected from diet-induced obesity and had improved glucose metabolism. On the other hand, inhibition of Ang-2 in obese mice not only decreased AT vascularity, but also enhanced AT inflammation and impaired glucose tolerance (43). Ang-2 expression in adipocytes is controlled by forkhead box C2 (FOXC2), overexpression of which also affects AT angiogenesis (44).

In accordance with a role of angiogenesis in regulating AT homeostasis, inactivation of Hypoxia-inducible factor-1α (HIF1α) in the AT resulted in exacerbated diet-induced obesity, insulin resistance and glucose intolerance, impaired heat generation and energy expenditure. Additionally, dysfunction of the brown AT together with reduced mitochondrial density was observed upon HIF1α inactivation in adipocytes (45). Hypoxia-inducible factor-2α (HIF2α) in adipocytes is also protective against obesity-related metabolic dysfunction. Deletion of this factor in adipocytes but not in myeloid or endothelial cells promoted insulin resistance in obesity, associated with white AT dysfunction and inflammation resulting from reduced vascularisation. Furthermore, obese mice with adipocyte-specific HIF2α inactivation displayed brown AT “whitening”, reduced UCP1 expression and decreased thermogenesis (33).

A further aspect demonstrating the intimate functional cross-talk between the vasculature and adipocytes in the AT is that PDGFRα-expressing adipocyte progenitors, which give rise to mature adipocytes, reside in the AT vasculature (46). Adipocyte progenitors are characterized by expression of the multi-zinc finger transcriptional regulator Zfp423 (47). Interestingly, besides perivascular adipocyte progenitors, some endothelial cells also express Zfp423, which might point to a potential role of vascular endothelial cells for adipogenesis (48).

Is therapeutic modulation of angiogenesis an approach to affect AT function and prevent obesity-related metabolic dysregulation? Although experiments targeting VEGF in adipocytes clearly suggest that this factor plays a protective role against obesity-induced metabolic dysfunction, systemic antibody-mediated inhibition of VEGF improved systemic glucose tolerance in obesity in mice, which was linked to improved hepatic insulin sensitivity (49). Interestingly, systemic administration of the angiogenesis inhibitor TNP-470 blocked genetic or diet-induced obesity and insulin resistance development in mice, associated with reduced vascularisation of the AT (50). On the contrary, a nanoparticle approach delivering either the PPARγ activator rosiglitazone or a prostaglandin E2 analog specifically to AT vasculature promoted AT angiogenesis and beige adipogenesis and ameliorated body weight gain and metabolic dysregulation in murine diet-induced obesity (51). It is therefore clear that systemic versus local, AT-specific manipulation of angiogenesis may have completely opposite actions on the outcome of obesity and obesity-related metabolic dysfunction, especially because systemic administration of angiogenesis modulators may affect further organs besides the AT. These controversies regarding the role of therapeutic modulation of angiogenesis in obesity and obesity-related complications have to be carefully evaluated and addressed in the future.

**Angiogenesis in diabetes and cancer:**

**PI3K signalling links metabolic control to endothelial function**

Common signalling cascade routes, which at the same time control metabolism and endothelial function, represent a potential link between dysfunctional angiogenesis and metabolic disorders. One such point of convergence is the PI3K pathway (52), a signalling cascade triggered both by metabolic factors like insulin and multiple different angiogenic factors like VEGF and FGFs.

PI3Ks are a family of lipid kinases that act downstream of most receptors to produce on plasma and internal membranes a secondary messenger phosphoinositides (PtdIns) phosphorylated on the position 3 of the inositol ring. Among the different 3-phosphorylated PtdIns, the best characterised is PIP3, a signalling molecule acting as a docking site for multiple proteins including Ser/Thr kinases like Akt, Tyr kinases like Btk, and regulators of small GTPases like the Rac activator P-Rex (53). PI3K-dependant activation of these elements modulates a plethora of cellular responses including proliferation and migration. For example, PI3K-activated Akt can regulate transcription through the inhibition of FOXO proteins nuclear translocation, modulate cellular metabolism and protein synthesis by activating the mTOR kinase, inhibit apoptosis by blocking BAD and promote proliferation by acting on p27/p21 cyclin-dependent kinase inhibitors (53). Furthermore, growth factors can trigger VEGF transcription through the PI3K-dependent stabilisation of HIF-1α (54).

Although the effects of PI3K activation converge to the same signal transducers, different receptors can preferentially activate one or few of the eight different PI3K isoenzymes encoded by the human genome. These eight distinct genes are further classified in three subfamilies (class I, II and III PI3Ks) and the four members of the class I group (PIK3CA, PIK3CB, PIK3CD, PIK3CG) have been extensively characterised in respect to endothelial cell functions (52). The product of the PIK3CA gene, Class I PI3Kα, acts as a dimer with members of the p85 family and responds to tyrosine kinase receptors of either VEGF or FGFR2, well-known regulators of angiogenesis. In line with this finding, PI3Kα is a key controller of angiogenesis and the loss of this enzyme in mice leads to an embryonic lethal phenotype in early gestation due to abnormal vascular development (55). Endothelial cells lacking PI3Kα show significant defects in sprouting and vascular remodelling, suggesting that this PI3K isoform is the main driver not only of proliferation but remarkably also of migration in response to angiogenic stimuli.
triggered by tyrosine receptor agonists. A potential explanation resides in the ability of PI3Ka to regulate small GTPases like RhoA and subsequently act on cytoskeletal remodelling (55).

Although the most potent angiogenic factors appear to signal through PI3KCA, the other class I PI3Ks can also play a role in endothelial cell function. While PI3Ka acts downstream of tyrosine kinase receptors, PI3KB and γ act downstream of G protein-coupled receptors (GPCRs) and show some redundancy in the response to GPCR agonists like, for example, S1-P or CXCL12/Sdf-1 (55–57). PI3KB can directly promote endothelial cell migration by acting on Akt but, on the other hand, PI3Ky specifically triggers Rac activation and actin cytoskeleton remodelling to direct endothelial cell motility (56). PI3Ky is also the key PI3K isoform that controls revascularisation after injury (57). This is likely explained by the fact that PI3Ky, similarly to PI3Kβ, is preferentially expressed in leucocytes and indirectly drives angiogenesis by controlling inflammation and the recruitment/differentiation of pro-angiogenic white blood cell populations (53, 58). Although the role of class II and class III PI3K in angiogenesis is currently poorly understood, the ubiquitously expressed PI3K–C2α is involved in VEGF receptor recycling and its ablation in mice causes severe vascular abnormalities that lead to an embryonic lethal phenotype (59). PI3K–C2α also plays a role in adult vasculogenesis and its connection to primary cilium function (60), a known cellular mechanosensor, suggests that it might be involved in shear stress sensing. Further studies are needed to understand more clearly how this enzyme controls blood 59ssel organisation and if this synergises with other PI3Ks.

The unexpected finding that hyperactive mutants of class I PI3Ka usually found in cancer also occur in congenital venous malformations further supports the observation that PI3K signalling is involved in vasculogenesis at various levels. Expression of the H1047R PI3KCA mutation, detected in a plethora of human cancers and hyper-proliferative disorders, in mice leads to the formation of vascular abnormalities due to endothelial cell hyperproliferation as well as impaired vessel maturation (61, 62). Strikingly, 30% of venous lesions from patients show the presence of the same mutation confirming that somatic mutation of PIK3CA is causally linked to such vascular malformations (61). In further agreement, other vascular abnormalities like lymphatic malformations show similar activating mutations of PIK3CA (63). While the systemic administration of PI3K inhibitors has been hampered by severe side effects, treatment of such vascular abnormalities appears to respond to either topical administration of PI3Ka inhibitors (61) or mTOR inhibitors like rapamycin that block the PI3K pathways downstream (62). While this opens unexpected applications of PI3K inhibition, these findings corroborate the view that PI3K signalling pathway is indeed a key controller of angiogenesis and vasculogenesis. In line with this view, targeting of PI3K signalling can represent a valuable route to inhibit angiogenesis. While most of the current treatments blocking angiogenesis usually intercept a single angiogenic stimulus, PI3K inhibition has the potential advantage of blocking several inputs that converge on the same pathway within the endothelial cell. Whether this can be exploited also to improve the efficacy of antiangiogenic therapies in cancer is still controversial (52) and awaits further studies.

The role of the PI3K signalling in regulating metabolism is emerging as a key to interpret the effects of PI3K inhibition. PI3K signalling triggered by key growth factors and hormones like VEGF and insulin, respectively, is known to lead to increased glucose uptake, lipid synthesis and protein translation. This is generally mediated by the ability of Akt to trigger the activation of the mechanistic target of rapamycin (mTOR), a key metabolic controller acting in the modulation of glucose and lipid homeostasis as well as protein synthesis (64). These activities are critically required in angiogenesis, whereby endothelial cells have to migrate, proliferate and differentiate in conditions of limited perfusion, in response to low oxygen tension or limited nutrient supply. Quiescent endothelial cells have to rapidly respond to these low energy conditions with an increase of biological functions. It is thus not surprising that the metabolism of sprouting endothelial cells is mainly based on glycolytic rather than aerobic catabolism (reviewed in (65)). Activated endothelial cells triggered to induce angiogenesis engage glycolysis and therefore appear to share some metabolic similarities with cancer cells that tend to rely on glycolysis, even in the presence of high oxygen (designated aerobic glycolysis) (66). This process, similar to the Warburg effect seen first in cancer cells, allows endothelial cells to produce intermediary metabolites required for the biosynthetic pathways. For example, the use of glucose through the pentose phosphate shunt pathway can contribute to the production of nucleotides precursors needed for nucleic acid production as well as reducing equivalents under the form of NADPH required to synthetise membrane lipids (67). These effects are clearly mediated by activation of receptors for angiogenic factors like VEGF, FGFs or chemokines like CXCL12 (67).

In addition, PI3K activation downstream of various receptors drives the glycolytic flux as well as the pentose phosphate switch (68). Given that the PI3K pathway is triggered downstream of both VEGF and FGF receptors, it is tempting to speculate that these metabolic changes are driven by PI3K activation. In line with this view, the highly motile tip cell at the top of the angiogenic sprout requires increased expression of the key regulator of the glycolytic flux, PFKFB3 (6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3), and expression of this key enzyme in endothelial cells is promoted by activation of PI3K signalling (69–71) (Figure 2). Furthermore, in proliferating endothelial cells of the stalk forming the base of sprouting newly formed vessels, VEGF triggers PI3K–dependent inhibition of the FOXO1 transcription factor (72, 73) and this leads to the consequent elevation of Myc expression, which potently triggers glycolysis, anabolic metabolism and growth (74). Conversely in quiescent endothelial cells of mature vessel walls, increased activity of FOXO1 leads to Myc inhibition and reduction of the metabolic rate both in terms of glycolysis and oxygen consumption (74). This appears to be in line with the notion that in quiescent endothelial cells the VEGF/PI3K pathway is generally in a low activity state, mainly supporting cell survival (75).
Therefore, endothelial cells must comply with a delicate balance of metabolic regulation. Consistently, systemic metabolic dysregulation is clearly a major driver of endothelial dysfunction. This is particularly important in diabetes, where hyperglycaemia resulting from insulin resistance or autoimmune destruction of pancreatic islets is a key determinant of the micro- and macro-vascular complications, including retinopathy, delayed wound healing, atherosclerosis and coronary artery disease (76). The endothelium is the primary cell exposed to and affected by the systemic effects of hyperglycaemia/hyperinsulinaemia in diabetes. Hyperglycaemia, for example, induces excessive production of reactive oxygen species (ROS), which in turn can indirectly disturb endothelial cell function. Excessive blood glucose disturbs the production from endothelial cells of the vasorelaxant agonist NO through uncoupling of the endothelial nitric oxide synthase (eNOS), which in turn leads to a vicious cycle, whereby NOS-produced ROS further reduce NO availability (77). In addition, advanced glycation end products (AGE) resulting from the non-enzymatic glycation of proteins or lipids in diabetes and other chronic disorders interact with their receptor RAGE on the endothelium triggering endothelial inflammation and dysfunction and thereby diabetic vascular complications (78–80). Furthermore, enhanced generation of superoxide in the diabetic endothelium may result in activation of a series of pathways, including enhanced polyol pathway flux or increased activity of the hexosamine pathway, as well as increased formation of AGE, thereby contributing to a vicious cycle of endothelial dysfunction (81, 82).

Hyperglycaemia is associated with the inability of the insulin receptor to induce glucose uptake. Given that insulin-induced translocation of the glucose transporter GLUT4 as well as the expression of GLUT1 is PI3K-dependent, this event is largely caused by the inability of insulin receptors to trigger the PI3K pathway (Figure 2), either because of lack of insulin (Type 1 diabetes) or due to insulin resistance (Type 2 diabetes). In endothelial cells, abnormal insulin signalling can directly disrupt endothelial cell function and further aggravate the diabetic condition. For example, insulin triggers eNOS activation, which produces the NO required to dilate vessels and improve glucose uptake in peripheral organs such as skeletal muscles. This process depends on insulin receptor-dependent PI3K activation that, in turn, triggers Akt-mediated phosphorylation and activation of eNOS (83). In addition, insulin and IGF-1 can signal to PI3K to stabilise HIF-1α and trigger the consequent VEGF expression as well as the glycolytic switch required for angiogenesis (54, 84). Genetic inactivation in mice of the insulin receptor or its downstream substrate IRS, coupling insulin to PI3K signalling, leads to emergence of vascular dysfunction, including impaired vascular tone regulation, and metabolic dysregulation (85, 86). In agreement, data from patients with rare genetic abnormalities in the insulin-dependent signalling to the PI3K pathway indicate that endothelial dysfunction is coupled with impaired ability of eNOS activation (87, 88).

In cancer, PI3K inhibition may have potentially beneficial effect in parts by acting in an anti-angiogenic fashion (89). On the contrary, PI3K inhibition in diabetes is likely to worsen diabetes-associated vascular disease (84). Targeting endothelial dysfunction in type 2 diabetes requires restoration of insulin sensitivity and normalisation of the PI3K/Akt/eNOS signalling cascade. Direct PI3K activators are clearly not safe as they might cause severe endothelial overgrowth (61, 62). However, alternative strategies targeting the metabolic pathways involved and/or further downstream effectors...
would be potentially relevant. To this end, a more comprehensive view of the signalling pathways and their impact on metabolic control as well as a better view of endothelial cell metabolism is required. Significant efforts are moving on that direction and future important developments are expected along this research line.

Conclusions and outlook

The role of deregulated angiogenic signalling in the development of vascular and metabolic disease is increasingly appreciated. Metabolic diseases, like diabetes, present often with systemic vascular complications that affect various organs and tissues, including the retina. Treatment options include anti-VEGF as well as other antiangiogenic modalities, including the manipulation of intracellular angiogenic signalling pathways, including the PI3K pathway. Yet, systemic effects of such treatments have to be evaluated and monitored carefully. In addition, vessel normalisation strategies that restore endothelial function and prevent vessel destabilisation may turn out as promising alternatives to the modulation of angiogenesis in the context of metabolic-vascular disease (90–92). In addition, modulating angiogenesis-regulating pathways, such as the PI3K pathway, may lead, in a context-dependent manner, to beneficial effects (e.g. in cancer) or detrimental effects (e.g. in diabetes) (84, 89). That therapeutic modulation of angiogenesis requires evaluation in a context-dependent manner becomes also evident from the recent results demonstrating the importance of angiogenesis in obesity and obesity-related metabolic dysregulation. Systemic inhibition of angiogenesis resulted in opposite results as compared to local manipulation of angiogenesis in the adipose tissue with regards to the outcome of obesity and insulin resistance (34, 49). Thus, the pros and cons of therapeutic modulation of angiogenesis in metabolic disease must be carefully evaluated in the future.

Acknowledgements

This work was supported by Telethon (GGP 14106), Associazione Italiana Ricerca Cancro (AIRC) (161813), Compagnia di San Paolo and World Wide Cancer Research Association (formerly known as AICR)(151324) to EH. TC was supported by the Deutscher Forschungsgemeinschaft (CH279/5–1 and SFB 655).

Conflicts of interest

EH is the founder of Kither Biotech SrL. All other authors declare no conflict of interest.

References
