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REVIEW

Trophoblast-mediated spiral artery remodelling: a role for apoptosis

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Abstract

In the first 20 weeks of pregnancy a number of important changes take place in the maternal uterine vasculature. Vascular endothelial and smooth muscle cells are lost from the spiral arteries and are replaced by fetal trophoblast cells. The resulting increase in blood flow to the intervillous space ensures that the fetus receives the nutrients and respiratory gases required for growth. Failure of the vessels to remodel sufficiently is a common feature of pregnancy pathologies such as early pregnancy loss, intrauterine growth restriction and pre-eclampsia. Although there is evidence to suggest that some vascular changes occur prior to trophoblast invasion, it is clear that in the absence of trophoblast invasion the remodelling of the spiral arteries is reduced. The cellular and molecular mechanisms by which trophoblasts influence vessel structure have been little studied. Trophoblasts synthesize and release a plethora of cytokines and growth factors including members of the tumour necrosis factor family such as tumour necrosis factor α, Fas-ligand and tumour necrosis factor-related apoptosis-inducing ligand. Recent studies suggest that these factors may be important in regulating the remodelling process by inducing both endothelial cell and vascular smooth muscle cell apoptosis.

Key words apoptosis; TRAIL; FasL; VSMC.

Introduction

Spiral arteries develop in the second half of the menstrual cycle under the influence of progesterone (Ferenczy et al. 1979). They arise from radial arteries at the endometrial/myometrial border. In the non-pregnant uterus they have muscular walls and a well-developed elastic lamina which diminishes as the artery penetrates the endometrium (Robertson & Warner, 1974). In the absence of an implanted blastocyst the vessels regress and are ultimately lost during menstrual shedding. Following implantation the spiral arteries progressively remodel during the first 22 weeks of gestation. From about week 10 the extent of remodelling correlates with cytotrophoblast invasion, with the greatest invasion and vessel transformation occurring within the central region of the placental bed and becoming less extensive towards the periphery (Pijnenborg et al. 1980). In the most heavily modified vessels extravillous trophoblast invade through the decidua and progress as far as the first third of the myometrium. In these vessels there is loss of a discrete muscle layer and the endothelium can be completely replaced by cytotrophoblast (Pijnenborg et al. 1980). At the placental periphery where invasion is less extensive both cytotrophoblasts and endothelial cells can be seen to co-exist within the vessel (Zhou et al. 1997). The loss of vascular cells is accompanied by fibrinoid deposition (Brosens et al. 1967; Kam et al. 1999) together with the loss of vascular function, most notably responsiveness to vasoconstrictors. The diameter of the resulting vessels can be increased at least 10-fold with the functional consequence being that the total blood delivered to the intervillous space is increased 3–4 fold but at a much reduced pressure (Thaler et al. 1990; Kliman, 2000). This ensures that the increasing demands of the growing fetus for nutrients, respiratory gases and for the removal of metabolic waste can be met. Failure or inadequate transformation of the spiral arteries is a common feature of pregnancy pathologies such as early pregnancy loss, intrauterine growth restriction (IUGR) and pre-eclampsia (PE).

Histological evidence would suggest that the remodelling that takes place within the decidual and myometrial segments of the spiral arteries occurs over a number of weeks (reviewed by Pijnenborg et al. 2006). However, although considerable advances have been made as a result of such studies they provide little insight into how these changes are regulated at a cellular and molecular

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level. A number of mechanisms may be responsible for the loss of vascular cells, including migration, dedifferentiation, phagocytosis/autophagy and apoptosis. These are not mutually exclusive, and it is likely that two or more precisely orchestrated processes will have a role to play. In this review we will discuss the recently emerging data suggesting that apoptosis of vascular cells may be involved in spiral artery remodelling and that this is, in part, mediated by trophoblast cells.

Decidual transformation: trophoblast-independent changes

The notion that changes in structure of the spiral artery occur prior to the arrival of the trophoblast has stimulated much debate. It is likely that priming of the vessels in preparation for invasion may be important for complete transformation to take place. Two studies performed by Craven et al. (1998) and Kam et al. (1999) tried to address these issues by comparing events that take place in normal and ectopic pregnancies. It is apparent from both these studies that mucosal decidualization in the absence of trophoblasts leads to changes in vessel structure. These changes included endothelial basophilia, vacuolation, increased endothelial activation and vessel dilation (Craven et al. 1998). However, complete 'physiological change' as first described by Brosens et al. required the presence of trophoblasts (Kam et al. 1999). The structural changes that occur prior to the arrival of trophoblasts have recently been termed decidua-associated remodelling (Pijnenborg et al. 2006).

The molecular mechanisms that drive decidual-associated transformation as a prelude to the more extensive remodelling that takes place following trophoblast invasion have been little studied. The sex steroids oestrogen and progesterone play a dominant role in regulating endometrial growth and regression (Rogers & Abberton, 2003) but it is not clear to what extent these hormones directly regulate either endometrial blood vessel growth or structure. The vascular effects of oestrogen are complex, and any effects on vessel remodelling are likely to be concentration- and time-dependent. Oestrogen is known to effect vascular reactivity (Huang et al. 2000) by stimulating nitric oxide synthesis. Oestrogen is also known to increase vessel permeability and endothelial cell proliferation through increased vascular endothelial growth factor (VEGF) release (Aberdeen et al. 2008).

There have been few studies on the direct effects of progesterone on uterine vessels, although it may influence vascular remodelling indirectly through its effects on the recruitment of immune cells such as lymphocytes, macrophages and uterine natural killer cells to the endometrium. For example, progesterone has been implicated in the regulation of leukocyte trafficking indirectly through its ability to up-regulate stromal cell chemokine expression (Sentman et al. 2004).

Trophoblast-dependent transformation

In humans, trophoblasts differentiate from cytotrophoblast stem cells along two pathways. Villous trophoblasts fuse to form a syncytium and are bathed by maternal blood, whereas extravillous trophoblasts are functionally defined by their invasive nature. The extravillous trophoblasts migrate from the anchoring villi to form two subpopulations. The interstitial extravillous trophoblasts invade the uterine wall, whereas the endovascular extravillous trophoblasts migrate along the lumen of the spiral artery in a retrograde manner as far as the myometrial segment. The source of these endovascular trophoblasts is still a topic of debate. Some advocate migration from the distal opening of the arteries while others favour trans-stromal migration and subsequent penetration of the arterial wall (Khong et al. 1986; Enders & King, 1991; Blankenship et al. 1993; Meekins et al. 1997; Coukos et al. 1998). Why the extravillous trophoblasts target the spiral arteries and arterioles and not the uterine veins (Pijnenborg et al. 1980, 1983) is unclear. Studies have shown that venous endothelial cells express the ephrin EPHB4 and it is suggested that this acts to repulse invading cytotrophoblasts (Red-Horse et al. 2005). Whether there is also a role for chemoattractants produced by the arteries needs examination. It has also been suggested that oxygen gradients could have a part to play in this intriguing biological phenomenon, a possibility that is discussed in more detail elsewhere (Cartwright et al. 2007).

Cardiovascular remodelling

The study of human spiral artery transformation during early pregnancy has been hampered by the lack of an appropriate model. In an effort to address this, we and others have looked for parallels in other systems of physiological and pathological remodelling. Vascular remodelling is a term used to describe the dynamic processes within the wall of blood vessels and involves complex interactions between cell growth, death, differentiation and migration, as well as extracellular matrix synthesis and degradation. Physiological vascular remodelling takes place in the perinatal lung and this period also sees significant changes in the abdominal aorta, a vessel that experiences a > 90% drop in blood flow following the loss of the placenta (Cho et al. 1995). Vascular smooth muscle cell (VSMC) death is also seen in the umbilical and uterine vessels. Pathologically, VSMC death is observed in late stage atherosclerosis, resulting in plaque instability (Clarke et al. 2008). In aortic aneurysm, localized structural deterioration of the aortic wall through loss of cells is known to lead to dilation and ultimately rupture (Lopez-Candales et al. 1997). Physiological and pathological states can also lead to positive vascular remodelling. In pulmonary hypertension, VSMC hyperplasia and hypertrophy results

in vascular wall thickening and a reduction in the luminal diameter (Tuder et al. 2007). This is also true of the early stages of atherosclerosis following endothelial cell damage. Although the mechanisms involved in these stimulatory scenarios are less likely to be directly involved in spiral artery remodelling, their negative regulation could be important.

There are a number of mechanisms resulting in cell death which may contribute to vascular remodelling within the cardiovascular system. Necrosis is normally restricted to pathological processes following tissue damage and is usually observed with an inflammatory response. Autophagy is a process involved in the breakdown and recycling of organelles and plays an important role in tissue homeostasis. Apoptosis is an energy-dependent process characterized by typical morphological and biochemical changes including cytoplasmic and nuclear condensation, DNA fragmentation, membrane blebbing and blistering, and finally the production of apoptotic bodies.

The balance between pro- and anti-apoptotic stimuli is critical in determining the fate of individual endothelial and vascular smooth muscle cells within a vessel lumen. Endothelial cells are exposed to pro-apoptotic stimuli including transforming growth factor β and members of the tumour necrosis factor (TNF) family and anti-apoptotic factors such as vascular endothelial growth factor (VEGF) and hepatocyte growth factor. In addition, attachment-mediated survival signals initiated through integrin–matrix interactions are known to play an important role in their survival, such that loss of adhesion induces a type of apoptosis known as anoikis. We propose that invading trophoblasts tip this balance away from survival towards cell death either directly via the paracrine release of apoptotic factors or indirectly by stimulating loss of cellular adhesion. As the cells of the vessel are in a steady state, loss of the endothelium together with the presence of the trophoblast could then induce vascular smooth muscle cell death.

Members of the TNF family of cytokines including TNF-α, TNF-related apoptosis-inducing ligand (TRAIL) and Fas-ligand (FasL) are important in the regulation of vascular cell apoptosis. Binding of these ligands to their appropriate receptors leads to trimerization and the activation of a number of pathways. TNF-α activates two distinct receptors: TNF-receptor 1 (TNFR-1) and TNFR-2. Both receptors are expressed by endothelial and vascular smooth muscle cells (Goetze et al. 1999). Binding of TNF to TNF-R1 leads to recruitment of the intracellular adapter molecule TNF receptor-associated death domain (TRADD), which has the ability to recruit a number of different proteins to the activated receptor. Recruitment of TRAF2 (TNF-associated factor 2) transiently activates the JNK pathway and promotes cell survival, whereas association with Fas activating death domain (FADD) stimulates pro-caspase 8 cleavage and apoptosis.

TRAIL signals through five independent receptors of which TRAIL-receptor 1 [TRAIL-R1, death receptor 4 (DR4)] and TRAIL-R2 (DR5) initiate apoptosis and are expressed on endothelial and vascular smooth muscle cells (Secchiero et al. 2003; Keogh et al. 2007). There are also two decoy receptors (DcR1 and DcR2), which are also expressed by endothelial cells from different vascular beds (Secchiero et al. 2003; Spierings et al. 2004; Chen & Easton, 2008). Binding of TRAIL to the decoy receptors does not induce apoptosis but may compete for TRAIL binding with TRAIL-R1 and -2 (Zhang et al. 2000).

The third member of the TNF family of cytokines that demonstrates apoptotic activity within the cardiovascular system is Fas-ligand (Fas-L). Fas-L binds to and activates the cell surface receptor Fas (CD95). The resulting receptor trimerization leads to the recruitment of FADD and pro-caspase 8 and following autoproteolytic processing, activated caspase 8 is released. In some cells, activation of caspase 8 is sufficient to induce apoptosis, whereas in others an amplification cycle is required whereby caspase 8 mediates cleavage of the pro-apoptotic Bcl-2 family member Bid. Proteolytic cleavage of Bid initiates the release of pro-apoptotic molecules from mitochondria such as cytochrome c.

Spiral artery remodelling

There are a number of ways by which endovascular and interstitial trophoblasts might influence spiral artery remodelling. In some spiral arteries there is evidence of smooth muscle cell reorganization, while medial necrosis and fibrinoid deposition have been observed (Kam et al. 1999). Extracellular matrix changes in decidual tissue have also been observed and, although primarily thought to be associated with trophoblast invasion, could also influence vessel wall structure by priming the vessel for subsequent trophoblast interaction (Aplin et al. 1988). Other mechanisms that may be involved include loss of adherence, migration, dedifferentiation and apoptosis. Clearly these processes are not mutually exclusive; for instance loss of endothelial cell adherence could lead to the form of apoptosis known as anoikis and changes in extracellular matrix composition could result in changes to the state of differentiation of the VSMC. The close relationship between endothelial cells and the underlying VSMC is central to maintaining a stable vascular structure. Loss of this relationship is likely to be crucial to the remodelling that takes place in the uterine spiral arteries.

In addressing the mechanisms that may be involved in vessel transformation the contribution of the two routes of extravillous trophoblast invasion should be considered. In this regard, interstitial trophoblasts are more likely to influence the behaviour of smooth muscle cells, whereas the endothelial cells are more likely to be the target, at least in the first instance, of endovascular invasion. It is also possible that the molecules and mechanisms involved in endothelial cell loss may differ from those employed to remodel smooth muscle cells.

The study of human spiral artery remodelling is restricted by the availability of material at all stages of gestation. the Animal models are amenable to manipulation at both the cellular and molecular level; however, direct extrapolation to the human situation is not always possible, as there are significant differences seen during spiral artery remodelling (Leonard et al. 2006). Similar loss of the endothelium and overall structural re-organization of the spiral arteries occurs in the mouse; however, comparison of different strains of immunodeficient mice indicates that uterine natural killer cells play a major role in remodelling of this species in the absence of trophoblasts (Greenwood et al. 2000). Over the last few years we have developed human cellular and *ex vivo* tissue models to address some of these issues. These, together with the technical advances that have been made in time-lapse microscopy techniques, have enabled some of these questions to be addressed.

Apoptosis and spiral artery remodelling

We have investigated the hypothesis that invading extravillous trophoblasts remodel spiral arteries by inducing vascular cell apoptosis. The asynchronous nature of apoptosis would fit with the gradual remodelling of the spiral arteries seen *in vivo.* As apoptosis, unlike necrotic death, does not induce an inflammatory response, there would be no collateral tissue damage.

Direct co-culture of both primary first trimester extravillous trophoblasts and the extravillous-derived cell line SGHPL-4 stimulated caspase-dependent apoptosis of both endothelial and vascular smooth muscle cells. Transwell co-culture experiments and incubation with trophoblast-conditioned medium indicated that this effect was, at least in part, mediated by soluble factors. Trophoblasts synthesize a number of candidate molecules which, in other settings, have been implicated in vascular cell apoptosis. These include TNF-α, FasL and TRAIL. Both FasL and TRAIL can be membrane-associated or soluble proteins. The soluble form of TRAIL is released following proteolytic cleavage of carboxy-terminus by cysteine proteases. Soluble FasL can be shed from the surface following the action of matrix metalloproteinases (MMPs) (Powell et al. 1999; Matsuno et al. 2001; Mitsiades et al. 2001) but whether sFasL inhibits or stimulates apoptosis is an area of some debate (Powell et al. 1999; Cunningham et al. 2005).

Our data using a blocking antibody to FasL/sFasL inhibited VSMC and endothelial cell (EC) death following culture with both trophoblast cells and also trophoblast-conditioned media, indicating a role for sFasL in this process (Ashton et al. 2005; Harris et al. 2006). Trophoblast-induced apoptosis of cells through the action of sFasL/FasL is not without precedence as it has been shown that trophoblast can induce apoptosis in T-lymphocytes, which may be important in regulating immune privilege at the fetal–maternal interface (Abrahams et al. 2004). TRAIL was originally thought only to induce apoptosis in transformed cells; however, it is now clear the many cells express TRAIL receptors and that TRAIL may have important physiological actions. Activation of both TRAIL-R1 and -R2 was involved in trophoblast-induced VSMC apoptosis (Keogh et al. 2007). A similar role for TRAIL in smooth muscle apoptosis induced by lymphocytes has been reported (Sato et al. 2006); however, in a recent study TRAIL stimulated smooth muscle cell proliferation (Kavurma et al. 2008). The explanation for this discrepancy is as yet unknown.

Perfusion of spiral arteries derived from term non-placental bed biopsies with first trimester extravillous trophoblasts or trophoblast-conditioned medium followed by static incubation for up to 72 h led to loss of both endothelial and smooth muscle cells (Ashton et al. 2005; Harris et al. 2006). This was inhibited by incubation with the caspase inhibitor zVAD-fmk and blocking antisera to FasL and TRAIL-R1, substantiating the effects observed *in vitro* (Ashton et al. 2005; Harris et al. 2006; Keogh et al. 2007). Confirmation of the effects of trophoblasts on vascular cell apoptosis *in vivo* have subsequently been reported in an interesting study where first trimester chorionic villi were transplanted to the mammary fat pads of Scid mice. After 3 weeks the authors reported specific endothelial and smooth muscle cell apoptosis in arterioles, although the mechanism of induction of apoptosis was not investigated (Red-Horse et al. 2006).

It is possible that a change in phenotype of vascular cells could alter their interactions with trophoblast and their sensitivity to apoptotic stimuli. VSMC can switch between 'functional' (contractile) and 'synthetic' (proliferative) phenotypes accompanied by changes in expression of multiple genes (Kaplan-Albuquerque et al. 2005). In pathological pregnancies such as those complicated by PE, failed transformation of spiral arteries in the placental bed is often accompanied by atherotic changes including neointimal proliferation of cells that are probably dedifferentiated VSMC (Pijnenborg et al. 2006). Plasticity of VSMC phenotype might permit local control of the degree of sensitivity to apoptotic stimuli, and hence the rate of vessel transformation (Su et al. 2006; Halka et al. 2008). Loss of the contractile phenotype is associated with altered vessel wall structure, including loss of the layered organization of VSMC, migration away from the lumen and loss of differentiation markers, as may be seen in other examples of vascular remodelling (Cai et al. 2004).

Regardless of the pathways and mechanisms by which apoptosis is initiated, the rapid and efficient removal of apoptotic cells within the spiral artery would permit significant cell deletion without tissue damage. One such mechanism known to be involved in the remodelling of other tissues is phagocytosis. Here apoptotic cells are engulfed either by their neighbours or by professional phagocytes, such as macrophages. Removal of dying cells before lysis prevents the release of potentially toxic or

immunogenic intracellular contents, and in keeping with this aim, apoptotic cells can be removed within 1–2 h (Fries et al. 2005). Phagocytosis is signalled by a number of molecules, one of these being the anionic phospholipid phosphatidylserine. This lipid is normally confined to the inner surface of the plasma membrane. Following induction of apoptosis phosphatidylserine is moved to the extracellular surface of the cell by the action of a flippase. The exposure of phosphatidylserine on the outside is a very early feature of apoptosis in a number of cellular systems and precedes the nuclear events (Martin et al. 1995). It is therefore likely that in this scenario, cell clearance from tissues may occur before the target cells have the chance to display other characteristics of apoptosis such as chromatin condensation or membrane blistering (Mower et al. 1994).

Within the context of the spiral artery, trophoblasts have been shown to be phagocytotic (Choy & Manyonda, 1998) and could play a significant role in removing the dead and dying cells from the vessel. Other cells within the vessel including both VSMC and endothelial cells possess similar activity and there are also large numbers of professional phagocytes within the decidua that could be involved. The proficient clearance of apoptotic vascular cells may explain why it has proven difficult to detect apoptosis within rapidly remodelling tissues using an immunohistochemical approach (Fries et al. 2005; Clarke & Bennett, 2006). However, recent studies of sections of first trimester decidua basalis have demonstrated apoptotic markers in both spiral artery VSMC and EC (Smith et al. 2008).

Conclusion

In conclusion, spiral artery remodelling plays a central role in establishing and maintaining a normal pregnancy. It is both spatially and temporally regulated and failure for this remodelling to occur normally may result in common pregnancy disorders such as recurrent pregnancy loss, pre-eclampsia and IUGR. In spite of the obvious importance, very little is known of the mechanisms responsible. We have reviewed the data suggesting that trophoblast-induced vascular cell apoptosis may play a role through both expression and release of members of the TNF superfamily. We further suggest that specific induction of apoptotic cell death would ensure that the controlled remodelling required for maintenance of vascular integrity can occur. Rapid removal by phagocytic cells would ensure that this crucial adaptive process occurs without significant tissue damage.

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