The regulation of nerve and blood vessel ingrowth in aneural and avascular intervertebral disc and articular cartilage

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Review

Cellular & Molecular

The regulation of nerve and blood vessel ingrowth in aneural and avascular intervertebral disc and articular cartilage

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Abstract

Introduction

This review will discuss the regulatory mechanisms of both innervation and vascularisation within normally aneural and avascular tissues, and how they may become altered in degeneration enabling new nerve and blood vessel formation which is hypothesised to be a source of pain.

Conclusion

Normal intervertebral discs and articular cartilage are the largest aneural and avascular tissues in the human body yet during intervertebral disc degeneration and osteoarthritis these tissues become increasingly vascularised by small blood vessels and innervated by peptide containing sensory nerve fibres. The mechanism by which this process occurs remains largely unknown. Published data suggests that various factors present within the healthy tissues such as aggrecan, chondromodulin and semaphorins may act as repulsive barriers to neurite and endothelial cell invasion. During degeneration however, the synthesis of these molecules becomes disrupted, potentially leading to vascularisation and innervation of the tissue.

Introduction

Innervation and vascularisation depend on the ability of growth cones on axons and endothelial tip cells on endothelial cells to guide them to their final destinations. This process is regulated by guidance molecules within the native tissue niche[1,2]. In many tissues, these guidance molecules regulate the entry of nerves and blood vessels, yet in disease states, these mechanisms may become disrupted leading to the inappropriate entry of both nerves and blood vessels which can generate unwanted affects[3,4,5,6]. Studies have shown that typically aneural and avascular tissues such as the intervertebral disc (IVD) and articular cartilage (AC) become increasingly vascularised by small blood vessels and innervated by peptide containing sensory nerve fibres, which are hypothesised to elicit pain[3,4,7,8,9,10]. Normal IVDs and AC are suggested to be the largest aneural and avascular tissues within the human body, and are composed of proteins and matrix components which are inhibitory to the ingrowth of both nerve and blood vessels[11,12,13].

This review aims to discuss the regulatory mechanisms of both innervation and vascularisation within normally aneural and avascular tissues, and how they may become altered in degeneration enabling new nerve and blood vessel formation, which is hypothesised to be a source of pain generation[3,4,5,6].

Discussion

The authors have referenced some of their own studies in this review. These referenced studies have been conducted in accordance with the Declaration of Helsinki (1964) and the protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed. All human subjects, in these referenced studies, gave informed consent to participate in these studies.

The intervertebral disc

The IVD is crucial for providing the structure and function of the spine, allowing movement and flexibility yet preventing hyperextension. IVDs are composed of three distinct anatomical regions; the nucleus pulposus (NP) which is constrained by the annulus fibrosus (AF) and cartilaginous end plates (CEPs)[14]. The highly specialized composition of the matrix within the IVD allows movement and offers resilience to compressive forces and loads.

Articular cartilage

AC is a specialized connective tissue covering bony surfaces permitting smooth frictionless movement of synovial joints, and allowing the joint to withstand pressure and weight-bearing activities. Components of AC are very similar to those found within the IVD in that the dense extracellular matrix (ECM) is composed of predominantly type II collagen and proteoglycans which allows AC to resist tensile and compressive forces.

Mechanisms of innervation and angiogenesis

In order to investigate how largely aneural and avascular tissues permit the entry and/or formation of nerves and blood vessels during disease states, this review will describe the factors present within healthy tissues which prevent nerve and blood vessel ingrowth.

Within both the IVD and AC, a number of repulsive factors exist which prevent nerve and endothelial cell ingrowth. In vitro proteoglycans, chondromodulin and semaphorins have been shown to inhibit neuronal and endothelial cell migration, all of which are expressed in vivo in the IVD and AC.

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Review

Prevention of innervation & vasularisation in Normal IVDs and AC

Proteoglycans and Glycosaminoglycans (GAGs)

Proteoglycans have been widely investigated for their inhibitory properties towards both neurite outgrowth and endothelial cell adhesion and migration by a number of groups worldwide[11,12,15]. In vitro studies demonstrated aggrecan isolated from IVD[11] and AC[12] inhibits endothelial cell adhesion, migration and spreading. Thus suggesting that the loss of aggrecan, a natural anti-angiogenic factor during degeneration[11] may lead to unopposed entry of blood vessels into these tissues.

Chondromodulin

Chondromodulin-I (ChM-I), a 25kDa glycoprotein is a novel growth regulating factor[16], which is important in the maintenance of avascular regions of AC and IVDs. Studies by Hiraki et al., concluded that ChM-I was an inhibitor of endothelial cell tube formation[17], in addition to this, recent studies by Miura et al., found that ChM-I inhibits chemotactic migration of endothelial cells by destabilising the actin cytoskeleton of lamellipodia extensions[18].

Developmental studies in mice have identified that ChM-I expression is induced during the process of chondrogenesis and occurs in conjunction with production of type II collagen[19]. Shukunami et al., demonstrated that the level of ChM-I expression was substantially reduced in calcified zones of AC[19]. During gestational periods, immunohistochemistry studies identified a high percentage of ChM-I expression in ECM and chondrocytes of the IVD, which decreased after maturation, suggesting that ChM-I may regulate the degree of vascularisation that occurs during development[20]. Interestingly, immunopositivity of NP cells for ChM-I increased with degree of IVD degeneration[20] suggesting that ChM-I attempts to reduce the threat of vascularisation during disease states.

Semaphorins

Semaphorins are present within both healthy IVDs and AC[13,21]. They comprise a large family of axonal guidance molecules which are either membrane bound exerting their effects locally, or secreted and these can exert effects over a long distance[22]. Axonal guidance molecules signal via receptor complexes which ultimately regulate growth cone morphology via alterations to the actin cytoskeleton[23,24,25]. The Semaphorins share a highly conserved 500 amino acid 'Sema' domain followed by a PSI (plexin semaphorin integrin) domain, and are further subdivided into 8 classes based on their C-terminal structures[26]. Class 3 semaphorins are among the most well characterised members and are the only soluble form found within vertebrates.

Semaphorins signal their response through two prominent semaphorin receptors; the neuropilins (NRP) and the plexins[27,28,29,30]. Whilst the majority of membrane bound semaphorins signal via plexins alone, class 3 semaphorins require the neuropilin as an obligate co-receptor generating a high affinity holoreceptor complex[30,31].

Class 3 Semaphorins (Sema3)

Sema3A has been studied for its role in axonal repulsion of both sensory and sympathetic neurons in chick[32] and humans[33]. De Wit et al., localised sema3A to axons and dendrites on cortical neurons together with NRP-1[34]. More recently sema3A has been identified within the IVD at both gene and protein level along with its receptors the neuropillins and plexins[13]. Tolofari et al., revealed that sema3A and sema3F were present

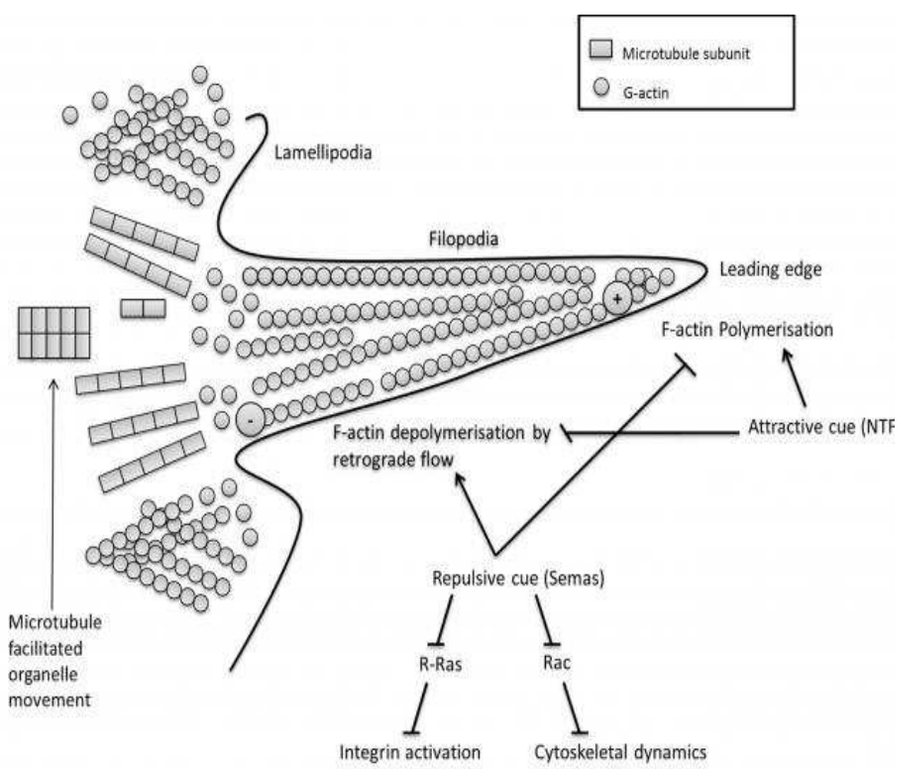

Figure 1: Schematic diagram of the regulation of axonal guidance of cells and their effects on directional growth concerning aneural and avascular tissue. The growth cone situated at the leading edge of the growing axon is able to sense and respond to cues within the environment. The growth cone is composed of lamellipodia which contains actin filaments and tensile structures composed of actin bundles known as the filopodia. In response to an attractive cue, F-actin is assembled and stabilised, whereas a repulsive cue would cause depolymerisation of F-actin, leading to the retraction of the filopodia.

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within the AF of healthy IVD tissue, whereas sema3A was decreased significantly in this region of degenerated discs[13]. Thus suggesting that the presence of sema3A within the healthy AF prevents the ingrowth of both nerves and blood vessels[13]. Sema3F has more recently been localised to the outer layers of the retina where it is thought to have vasorepulsive properties towards retinal and choroidal capillaries, as levels are seen to deplete in patients with pathologic neovascularisation of the outer retina[35]. Class 3 semaphorins are unique in that sema3B and sema3C exert bifunctional activity, and are able to act as repulsive or attractive cues, and block the collapsing action of sema3A on dorsal root ganglia (DRG) neurons[36].

Interactions between semaphorins and the cytoskeleton

During neuronal development, the axonal growth cone responds to various guidance cues within the surrounding environment. The growth cone selectively stabilises or destabilises the actin cytoskeleton in lamellipodia and filopodia to achieve directional growth[37] (Figure 1). The mechanism by which semaphorins interact with the cytoskeleton is a continued area of research, so far it has been reported that semaphorins alter the neuronal cytoskeleton by causing depolymerisation of F-actin[23,24,38]. Various signalling proteins are linked to semaphorin induced cytoskeletal changes within neuronal cells including the members of the Rho family of small GTP-binding proteins[39,40,41,42], collapsin response mediator proteins (CRMPs) and intracellular protein kinases. Plexins within the holoreceptor complex generate an intracellular response to semaphorin binding, ultimately resulting in altered cytoskeletal dynamics and cell migration. Plexins have intrinsic GAP (GTPase activating protein) activity that activates R-Ras, a GTPase responsible for sustained integrin activation[43,44]. Activation of R-Ras enhances focal adhesion formation, cell adhesion and cell spreading, as well as favouring the activation of PI3K[45]. The binding of semaphorin to plexin receptors on the surface of growth cones therefore inactivates R-Ras which prevents integrin mediated cell adhesion, blocking downstream PI3K. Additionally, semaphorin-plexin interaction also activates other pathways such as Rac and Rho resulting in the depolymerisation of actin and endocytosis (Figure 2).

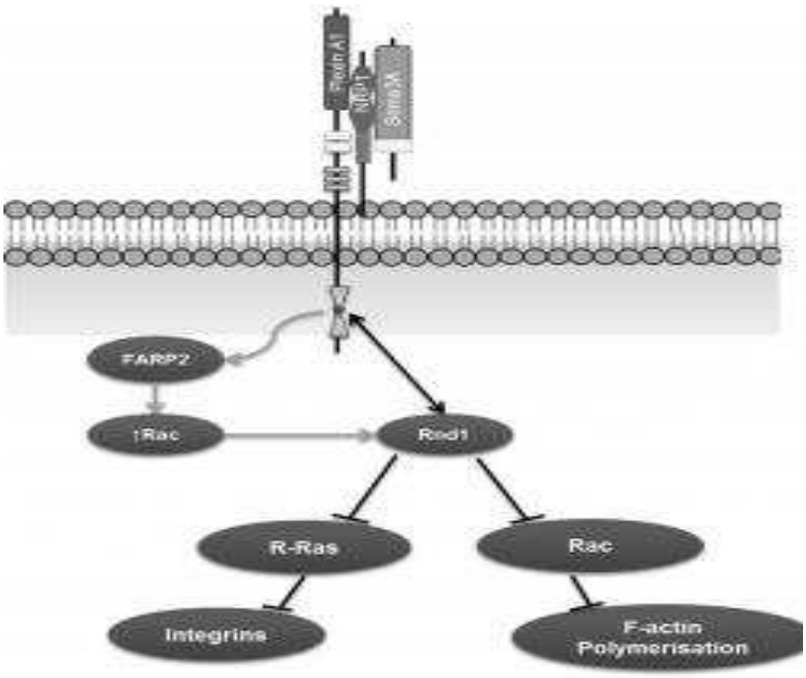

Figure 2: Semaphorin receptors and their intracellular signalling pathways. Semaphorin receptors can cause activation of a number of different intracellular signalling pathways [155]. Semaphorins can regulate integrin function and cytoskeletal alterations via the activation of R-Ras GAP activity of plexins. The binding of semaphorins to holoreceptor complex formed by NRP ligand and plexin signal transducing element leads to the dissociation of FARP2 from plexinA1 leading to an increase in Rac activity, this allows the association of Rnd1 and

Ben-Zvi et al., demonstrated that sema3A application induced cell death of NGF, BDNF and NT3 dependant DRG neurons, and identified the mechanism was through the phosphorylation of c-jun/JNK pathway [100]. Repulsive agents arrest or completely collapse growth cones and neurite outgrowth[38], as shown for sema3A which results in a loss of focal adhesions shortly followed by contraction of the cytoskeleton within endothelial cells[46]. A recent study by Yu et al., demonstrated sema3A inhibited both migration and tube formation of HUVECs and significantly inhibited phosphorylation of the JNK and P38MAPK signalling pathway suggesting effects on vascularisation as well as innervation[47]. Neural connectivity and path finding is reliant on axonal guidance cues which either act as attractants or repellents on growth cones and endothelial tip cells during neovascularisation and innervation, causing the filopodia and lamellipodia to turn towards or away from the stimulus achieving directional growth (Figure1).

Alternative roles of semaphorins in the regulation of angiogenesis

Extensive studies of class 3 semaphorins have revealed their involvement in several non-neuronal processes such as angiogenesis [35,48,49,50],organogenesis[51,52,53], tumorigenesis[50,54,55,56,57], immune cell function[58] and more recently a suggested role in osteoprotection[59] suggesting they may elicit a pleothora of actions within these tissues.

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Review

Angiogenic Factors

Several angiogenic growth factors have been localised to AC such as vascular endothelial growth factor (VEGF)[60], fibroblastic growth factor (FGF-2)[61] and transforming growth factor beta (TGFβ)[62] yet AC and IVD are unique in that they remain largely avascular. The process of angiogenesis is complex and includes local degradation of the basement membrane, migration and proliferation of endothelial cells followed by the formation of new capillary sprouts. Various molecules have been studied for their potential anti-vascular properties such as elastase and proteinase inhibitors which ultimately prevent the degradation of the basement membrane. Tissue inhibitors of metalloproteinase-1 (TIMP-1) and TIMP-2 have also been speculated to be involved in preventing angiogenesis[63], due to the inhibition of MMPs and thus decreasing matrix degradation.

In addition, although sema3A is classically known to repulse axonal growth cones[32] and cause apoptosis of NGF dependant sensory neurons[64] via the activation of JNK/c-jun signalling pathways[65], sema3A has now been shown to have similar effects on endothelial cells[46].

Other regulators of innervation and angiogenesis

ECM molecules located in both AC and the IVD have been studied for their role in angiogenesis. Proteoglycans found within the IVD and AC ECM such as decorin are known to inhibit endothelial cell migration, tube formation and VEGF production[15]. Thrombospondin-I (TSP-I) exerts similar effects on endothelial cell formation[63], and it is known that decorin binds to TSP-1 and potentiates the ability to block tube formation[66]. Various other anti-angiogenic molecules have been identified within AC and IVD such as troponin 1[67] angiostatin and endostatin[68]. Endostatin is a 20kDa c-terminal fragment of collagen type XVIII, first identified by O Reiley et al., from haemangioendothelioma in mice[68] and has since been recognised for its ability to inhibit VEGF induced endothelial cell migration[69] and neovascularisation[70].

Pufe et al., was the first group to identify the presence of endostatin within human cartilage[71]. Endostatin blocks angiogenesis by directly interacting with VEGFR2[72] which have been identified on the surface of hypertrophic and osteoarthritic chondrocytes[73,74,75].

Endostatin has also been shown to inhibit VEGF induced activation of MMP-1 and -2 from endothelial cells[76] and AC[77,78].

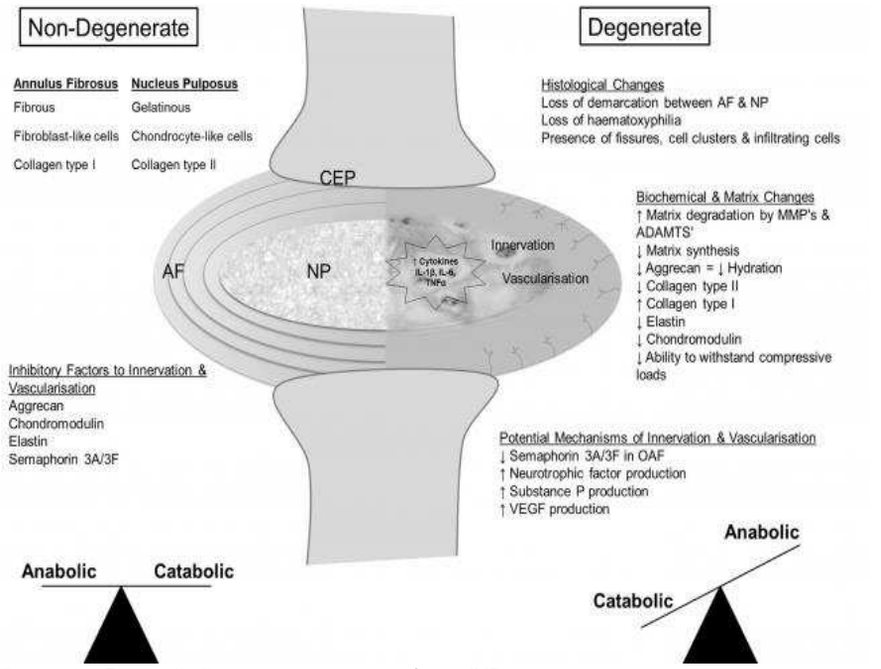

Figure 3: Figure 3: Schematic diagram of the IVD in a non-degenerate and degenerate state. In a non-degenerate "healthy" IVD, both anabolic and catabolic processes are balanced, and various factors are present which help maintain the aneural and avascular environment of the IVD. These include aggrecan which is abundant in the healthy NP, along with semaphorins 3A and 3F which are localised to the outer AF. Elastin and chondromodulin are also present within the NP and AF and have been shown to have vasorepulsive roles towards endothelial cells. In a degenerate state, anabolic and catabolic processes are imbalanced in favour of catabolic processes which cause a decrease in matrix synthesis and an increase in matrix degradation, this process is enhanced by the action of proinflammatory cytokines known to regulate factors such as MMP's and ADAMTS enzymes which subsequently leads to a decrease in aggrecan and thus dehydration of the NP. Semaphorin 3A and 3F are significantly reduced within the degenerate outer AF which would create a permissive environment for the entry of both nerves and blood vessels.

Disease mechanisms which lead to innervation and angiogenesis

There are various mechanisms within the normal IVD and AC which are thought to prevent the ingrowth of nerves and blood vessels. During disease states, these processes become disrupted which is suspected to allow the inappropriate entry of both nerves and blood vessels; ultimately leading to pain generation.

Characteristic changes in disease

The human IVD and AC undergo major biochemical alterations during degeneration which cause disruption to matrix homeostasis leading to the decreased synthesis of both collagen type II and proteoglycans, which are key features of degenerative processes[79,80]. Additionally,

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components which inhibit nerve and blood vessel ingrowth such as chondromodulin and semaphorins are also lost during degeneration which may lead to the innervation and vascularisation of the tissue[13,20].

Upregulation of matrix degrading enzymes

Early stages of degeneration are characterised by the imbalance of catabolic and anabolic processes[81], which ultimately results in the loss of matrix components vital to the structure and function of both IVD and AC tissues. Cytokines, particularly IL-1β and TNFα have been implicated to mediate the destruction in OA[82,83] and IVD degeneration[84,85,86,87,88,89,90,91,92,93]. These pro-inflammatory cytokines are known for their potent activity in increasing the production of matrix metalloproteinases (MMPs) and a disintergrin and metalloproteinase with thrombospondin-motif (ADAMTS) from chondrocytic cells of AC and IVD[85,86,94,95], leading to loss in normal matrix, facilitating the unimpaired ingrowth of nerves and blood vessels.

Loss of proteoglycans and collagen type II

The loss of proteoglycans and collagen type II is one of the earliest changes identified during degeneration, characterised by a switch in the type of proteoglycans and collagens synthesised. A decrease in aggrecan production is replaced by biglycan, decorin and versican[96,97], along with the switch in production of collagen from type II to type I[98] leaving the tissue dehydrated and unable to withstand compressive loads and forces.

Proteoglycans are known for their inhibitory effects on neurite ingrowth and so their presence within the healthy IVD and AC is pivotal not only for the structure and function of the tissues, but also in regulating the aneural and avascular environment[11]. As shown by Johnson et al., and Bara et al., aggrecan derived from the IVD and AC is able to prevent cell adhesion and migration[11,12], thus endothelial cell adhesion would be prevented in the normal disc and so NGF expressed by endothelial cells would not be able to enhance the survival of incoming neuronal cells.

Altered expression of inhibitory factors during degeneration

ChM-I is a matrix component of both AC and IVD known for maintaining the avascular environment, however during degeneration ChM-I is seen to decrease. Hayami et al., identified the presence of ChM-I within healthy avascular adult AC, and found that in early OA, levels of ChM-I decreased slightly within the superficial zone, yet in advanced OA, ChM-I was decreased in all zones of AC alongside an increase in the number of VEGF expressing chondrocytes[99].

Immunohistochemistry identified the localisation of incoming vascular endothelial cells within close proximity to VEGF expressing cells where there was lowered expression of ChM-I.

Semaphorins are thought to act as a barrier to neural and vascular ingrowth within healthy IVD and AC; this was suggested by Tolofari et al., who identified the presence of sema3A within the outer AF in healthy IVDs[13].

During degeneration however, sema3A was significantly decreased within the AF and immunopositivity increased around cell clusters within the degenerate NP. Invading nerves and blood vessels can be seen entering regions within the outer AF[13], suggesting that during degeneration, the loss of the inhibitory semaphorin barrier allows inappropriate vascularisation and innervation.

Okubo et al., also identified an overexpression of sema3A within osteoarthritic cloned chondrocytes a feature associated with degeneration. The increase in sema3A within AC was also shown to inhibit VEGF induced chondrocyte migration by competitive binding to NRP-1[21].

Inappropriate entry of nerves and blood vessels

Pain felt by patients with OA is sometimes described as a burning pain, which is characteristic of the pain generated by the presence of fine unmyelinated nerve fibres[100]. Mechanisms concerning the entry of nerve and blood vessels into usually avascular and aneural tissue is still incompletely understood. Various studies have reported the presence of cytokines: IL-1β and TNFα, which are potent pro-inflammatory mediators present in IVD degeneration[84,85,88,89,92,101,102,103] and OA[82] which cause disruption to the ECM composition.

The majority of sensory nerve fibres within IVDs and AC are associated with blood vessels, as they grow along endothelial cells after angiogenesis has occurred[104,105]. Freemont et al., were the first to identify the presence of small unmyelinated peptide-containing sensory nerve fibres and microvessels within degenerate lumbar IVDs of patients experiencing pain[4].

Later studies by the same group[3] observed microvessels expressing the neurotrophic factor NGF, whilst accompanying nerves expressed the high affinity receptor Trk A. Early observations identified neuronal growth alongside endothelial cells. Neurotrophic factors NGF, BDNF and NT3 and their receptors, the tyrosine kinases are involved in the survival of neurons and have been identified at gene and protein level within IVD and AC[106,107,108,109,110,111,112]; Recently Krock et al., demonstrated NGF and BDNF protein production by IVD organ cultures from degenerate disc were significantly higher than those from healthy discs[113] this agreed with previous studies which had shown increased levels of NGF expression in surgical degenerate IVD compared to postmortem tissues[111,114].

In contrast Purmessur et al., found high levels of NGF and BDNF within IVDs, with no significant changes between regions of the IVD and disease severity[109] demonstrating this area requires further investigation.

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Inflammatory cytokines present within the degenerate IVD and AC have been shown to regulate the expression of these neurotropic factors[106,108,109,112,115]. NGF dependant neurons are known to synthesise pain related peptides, substance P and calcitonin gene related peptide (CGRP)[10,114,116,117,118] leading to pain sensitisation, and these have been shown to be regulated by cytokines[109].

Conclusion

To conclude, there are numerous native molecules which exert inhibitory effects towards neural and vascular ingrowth within healthy non-degenerate IVDs and AC (Figure 3). Whilst during degeneration the depletion of these inhibitory factors and production of nerve and endothelial growth factors (Figure 3) leads to the inappropriate entry of nerves and blood vessels.

Authors' contribution

ALA Binch contributed to study design and drafted the manuscript. Alison K Cross and Christine L Le Maitre conceived the topic of the review, secured funding and critically revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

Abbreviations

AC: Articular Cartilage
ADAMTs: A Disintigrin and Metalloproteinase with Thrombospondin motifs
AF: Annulus Fibrosus
BDNF: Brain Derived Neurotrophic Factor
CEP: Cartilaginous End Plate
CGRP: Calcitonin Gene Related Peptide
ChM-I: Chondromodulin-I
CRMPs: Collapsin Response Mediator Proteins
DRG: Dorsal Root Ganglia
ECM: Extracellular Matrix
FGF-2: Fibroblast Growth Factor
GAGs: Glycosaminoglycans
GAP: GTPase Activating Protein
HUVEC: Human Umbilical Vein Endothelial Cells
IL-1: Interleukin 1
IVD: Intervertebral Disc
MMP: Matrix Metalloproteinase
NGF: Nerve Growth Factor
NP: Nucleus Pulposus
NRP-1: Neuropilin 1
NT3: Neurotrophin 3
OA: Osteoarthritis
PSI: Plexin Semaphorin Integrin
Sema3: Class 3 Semaphorin
TGFβ: Transforming Growth Factor Beta
TIMP: Tissue Inhibitor of Metalloproteinase
TNF: Tumour Necrosis Factor
Trk: Tropomyosin Kinase
TSP-I: Thrombospondin-I
VEGF: Vascular Endothelial Growth Factor
VEGFR2: Vascular Endothelial Growth Factor Receptor 2

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