CHAPTER 7

ANGIOGENIC RESPONSE OF ENDOTHELIAL CELLS TO HEPARIN BINDING DOMAIN OF FIBRONECTIN*
7.1. Introduction

As discussed before, various proteolytic fragments of the multi-domain protein, FN have been shown to have activities not found in the intact protein. The cell binding domain of FN along with heparin binding domain has been reported to be important in forming focal adhesions and mediating cell adhesion and cell migration. Although the interaction of the cell binding domain of FN with integrin has been shown to regulate angiogenesis (Kim et al., 2000), the biological role of other domains of FN in the process of angiogenesis is not clear. The heparin binding domain (HBD) of FN is one such structural domain of interest as it influences cell adhesion and spreading by interacting with cell surface heparan sulphate proteoglycans (HSPGs) (Tumova et al., 2000; Wilcox-Adelman et al., 2002). Syndecans, a group of cell surface HSPGs have been shown to act in conjunction with integrins in promoting cell spreading and migration on FN matrix (Wilcox-Adelman et al., 2002). The present study was aimed to analyse the role of the heparin binding domain of fibronectin in the process of angiogenesis. Results of the experiments carried out using primary cultures of HUVECs maintained on FN, HBD and polylysine substrata and in vivo assay using CAM are discussed in this chapter.

7.2. Methods

FN was subjected to trypsin digestion and HBD was purified. The purity of isolated HBD was checked using SDS-PAGE followed by silver staining which showed a single band of ~33 kDa. HUVECs were isolated by collagenase perfusion and were maintained in culture on culture dishes passively coated with polylysine, FN or HBD of FN and analysed the attachment. The levels of angiogenic markers were assessed by ELISA. The activity of COX was determined oxygraphically. The mRNA levels of VEGF, VEGFR2 and COX-2 in cells maintained on FN, HBD and polylysine were studied using RT-PCR. The angiogenic potency of HBD, FN and the affinity isolated VEGF was tested in CAM assay. The level of post-translational modification of VEGF was analysed by western blotting. Solid phase binding and dot blot assays were used to assess the binding of VEGF to FN and HBD. The materials and methods used in these experiments are discussed in detail in chapter 2.

7.3. Results

7.3.1. Attachment of HUVECs to FN and HBD of FN

The ability of HUVECs to interact with the heparin binding domain of FN was confirmed by analyzing their attachment to HBD coated substrata (Figure: 7.1A.). Nearly 70% of the cells attached to HBD coated plates, which was not significantly different from that to FN at the concentration tested (20 µg/ml each). Addition of GAGs caused inhibition in the attachment (Figure: 7.1B.). Of the different GAGs tested, the maximum inhibition of attachment was caused by heparin followed by CSA. The inhibitory effect of heparin was significantly more on the attachment of cells to HBD (65%) than to FN (25%) suggesting that cells interact with the HBD possibly through heparin like molecules on the cell surface.

Figure: 7.1. Attachment of HUVECs: A) ECs (1.5 x 10^6 cells/ml) were seeded on plates passively coated with 20 µg/ml of FN or HBD. After incubation at 37°C for 1 hour, the unattached cells were removed, attached cells were harvested and the percentage attachment was determined. B) ECs were seeded on plates passively coated with 20 µg/ml of FN or HBD in the presence of 100 µg/ml each of hyaluronic acid (HA), chondroitin sulphate A (CSA) or heparin and incubated the cells for 2 hours at 37°C. The attachment was expressed as percentage of the respective controls. *Statistically significant compared to respective controls (p<0.05)
7.3.2. Pro-angiogenic effect of HBD

7.3.2.1. Effect of HBD on angiogenesis in CAM:- To study the angiogenic effect of intact FN and its heparin binding domain in vivo, 10μg of the proteins in PBS was applied on to the chorio allantoic membrane (CAM) of 4 day old chick embryo; PBS treated CAM served as the vehicle control. On the 12th day, the vasculature of the treated CAMs were photographed and hemoglobin was estimated as a measure of vessel density. A significant increase in the vessel density in CAMs treated with HBD of FN when compared with intact FN and the vehicle control was observed (Figure: 7.2.). A two fold increase in the hemoglobin level was observed in HBD treated CAM as compared with the FN treated ones.

![Figure 7.2. Pro-angiogenic effect of HBD in CAM assay:- 4 days old chick embryo CAMs were treated with 10 μg of FN, 10 μg of HBD (H) and non-matrix control polylysine (P). The CAMs were isolated after 8 days. Vascularisation in the CAM and the level of hemoglobin as a measure of the vessel density less vehicle control are given. The results presented are the average of three sets of experiments done in duplicate ± SEM. The photographs given are from a representative experiment. *Statistically significant compared to P (p<0.05)
**Statistically significant compared to P and FN (p<0.05)
7.3.2.2. Effect of HBD in rat aortic rings: - The angiogenic effect of HBD of FN was also tested in organ culture model of angiogenesis, rat aortic ring assay. A significant increase in the sprout density in aortic rings treated with HBD of FN when compared with intact FN (10 μg each) and the vehicle control was observed (Figure: 7.3.).

Figure: 7.3. Pro-angiogenic effect of HBD in aortic rings:- 1mm thick rat aortic rings were treated with 10 μg of FN (B) and 10 μg of HBD (C). Aortic rings treated with PBS served as the vehicle control (A). Arrows indicate sprout formation. (Magnification - 4x)

7.3.2.3. In vitro effect on HUVECs in culture: - The angiogenic nature of HBD of FN was further tested using in vitro culture of HUVECs. Isolated HUVECs were maintained on plates passively coated with FN, HBD and polylysine in serum free MCDB 131 medium and the morphological changes were analyzed

Figure: 7.4. Pro-angiogenic effect of HBD in vitro:- HUVECs were maintained in culture on plates passively coated with polylysine (a), FN (b) or HBD (c). The morphological changes were photographed on the first (1) and second day (2) under an inverted microscope (40x). The photographs given are from a representative experiment. Arrows indicate capillary-like structures.
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microphotographically (Figure: 7.4.). By 48 hours of culture, the cells on HBD showed grouping and formation of capillary-like structures whereas the cells on FN showed only grouping during this period. The cells seeded on polylysine did not show any significant morphological changes and remained spherical during this time.

The production of EC specific markers viz. Factor VIII antigen/von Willebrand Factor (vWF) and EC inducible function i.e. E-selectin and CD31 (PECAM 1) were also analyzed (Figure: 7.5.). The amount of vWF produced by cells was significantly greater in cells maintained on HBD than that on FN or polylysine (Figure: 7.5A.). Cells maintained on FN and HBD showed about 2.3 – 2.7 fold increase in the production of E-selectin compared to cells on polylysine coated plates during 48 hours of culture (Figure: 7.5B.). The kinetics of production of CD31 was also similar with no significant difference in the first 24 hours (Figure: 7.5C.). The level of CD31 was significantly high in cells maintained on HBD of FN compared to cells on polylysine after 48 hours in culture. The level of CD31 was 1.9 fold higher in cells maintained on HBD than those maintained on FN substratum.

**Figure: 7.5. Pro-angiogenic effect of HBD: Changes in markers of angiogenesis:-** The levels of vWF (A), E-selectin (B) and CD31 (PECAM1) (C) in cells maintained on FN, HBD (H) and polylysine (P) substrata were estimated by ELISA. Values given are the average of three sets of experiments done in duplicate ± SEM.

*Statistically significant compared to polylysine (p<0.05)

**Statistically significant compared to polylysine and FN (p<0.05)
7.3.3. Expression of VEGF and its receptors by HUVECs - Effect of HBD

To study whether the angiogenic nature of HBD was due to a change in the level of VEGF, the mRNA expression patterns of VEGF and its receptors VEGFR1 and R2 were studied by semi quantitative RT-PCR (Figure: 7.6.). The expression of mRNA of VEGF and VEGFR2 in cells maintained on matrix protein substrata were significantly more than those in polylysine coated plates and was in the order HBD > FN > polylysine. There was no detectable expression of VEGFR1 in cells maintained on different substrata.

**Figure: 7.6. Expression of VEGF and its receptors - RT-PCR - Effect of HBD:** HUVECs were maintained in culture for 48 hours on polylysine (P), fibronectin (FN) and HBD (H) coated culture dishes. Cells were harvested, total RNA isolated and mRNA levels of VEGF and VEGFR2 were analyzed by RT-PCR. The PCR products were separated on 1.75% agarose gel and bands visualized by ethidium bromide staining (A). The gel given is from a representative experiment. Relative intensity of VEGF (B) and VEGFR2 (C) bands were quantitated by BioRad Quantity One 4.5 software and normalized with that for internal control (GAPDH). Values given are the average of three sets of experiments done in duplicate ± SEM.

*Statistically significant compared to polylysine (p<0.05)
**Statistically significant compared to polylysine and FN (p<0.05)
7.3.4. Production of VEGF by HUVECs maintained on HBD

VEGF secreted by HUVECs maintained on different substrata was quantitated by ELISA (Figure: 7.7). The amount of VEGF was significantly high in the media from cells maintained on FN and HBD as compared to those on polylysine control.

![Figure: 7.7. Production of VEGF by HUVECs: Effect of HBD:- HUVECs were maintained in culture for 48 hours on polylysine (P), fibronectin (FN) and HBD (H) coated culture dishes. The media were collected, VEGF was quantitated by ELISA. The results presented are the average of three sets of experiments done in duplicate ± SEM. *Statistically significant compared to polylysine (p<0.05)

7.3.5. Effect of Calphostin C on angiogenic markers

In order to examine whether binding of cells to HBD causes intracellular effects through the protein kinase C dependent signaling pathway, which is known to be activated by cell surface syndecans, the effect of inhibition of PKC on expression of angiogenic markers was studied. The mRNA expression of VEGF and its receptors was analyzed in presence of protein kinase C (PKC) inhibitor, calphostin C (10 µM) in cells maintained on HBD and FN (Figure: 7.8.). The expression of VEGF was not significantly different from that of the control whereas the expression of VEGFR2 was significantly low in cells maintained on HBD treated with calphostin C as compared to untreated control. But the mRNA expression of VEGF and VEGFR2 did not show any significant change in cells maintained on FN and treated with calphostin C as compared to the controls.
Figure: 7.8. Expression of VEGF and its receptors – Inhibition by Calphostin C: HUVECs were maintained in culture for 48 hours on FN and HBD coated culture dishes with (H+C, FN+C) or without (H, FN) 10μM Calphostin C. Cells were harvested, total RNA isolated and mRNA levels of VEGF and VEGFR2 were analyzed by RT-PCR as described in legends to figure 7.6. The gel given is of a representative experiment (upper panel). Relative intensity of VEGF (A) and VEGFR2 (B) bands were quantitated and normalized with that for internal control (GAPDH). The results presented are the average of three sets of experiments done in duplicate ± SEM. *Statistically significant compared to HBD (p<0.05)

The protein levels of vWF, E-selectin, CD 31 and VEGF were also analyzed in the presence of calphostin C (Figure: 7.9.). The results showed that the levels of EC specific marker as well as angiogenic markers were significantly reduced on treatment with PKC inhibitor. The VEGF protein secreted into the medium also decreased significantly in cells maintained on HBD treated with the inhibitor when compared to untreated controls.
7.3.6. Angiogenic activity of affinity isolated VEGF from cells maintained on HBD

Angiogenic activity of heparin-sepharose affinity isolated VEGF secreted by cells seeded on FN, HBD and polylysine was analyzed by CAM assay (Figure: 7.10.) using equivalent amounts of VEGF. The hemoglobin levels were about 3.5 fold higher in CAMs treated with VEGF isolated from media of cells maintained on FN and HBD as compared to medium of cells on polylysine substratum.

To study whether VEGF binds to HBD of FN and if so it was biologically potent in inducing angiogenesis, VEGF isolated from medium of cells maintained on polylysine substratum was allowed to bind to wells coated with FN and HBD and was eluted with 1.5M NaCl in Tris-HCl. Equivalent amounts of VEGF as determined by ELISA were applied onto CAM to check its angiogenic potency (Figure: 7.10.). The vessel density in terms of hemoglobin was significantly high in CAM treated with VEGF bound to HBD showing its greater angiogenic potency. Wells treated with FN and HBD without treating with VEGF were also eluted with 1.5M NaCl and applied onto CAM as controls. But there were no significant difference in their hemoglobin content and vessel density as compared to the vehicle control.
Figure 7.10. Angiogenic potency of VEGF - Effect of HBD: 4 days old chick embryo CAMs were treated with equivalent amounts of VEGF, affinity isolated from media of cells maintained on polylysine (B), FN (C) and HBD (D) and the CAMs were isolated after 8 days. CAMs treated with TBS served as the vehicle control (A). The equivalent amounts of VEGF eluted from FN (E) and HBD (F) were also applied onto CAM. Wells treated with FN (G) and HBD (H) without treating with VEGF were also eluted and applied onto the CAMs. The topmost panel shows photographs of vascularised CAMs and below is given the levels of hemoglobin, less the vehicle control. Values given are the average of three sets of experiments done in duplicate ±SEM.

*Statistically significant compared to polylysine (p<0.05)
#Statistically significant compared to FN bound VEGF (p<0.05)
7.3.7. Analysis of post-translational modification of VEGF in cells maintained on HBD

The PAR modification of VEGF secreted by HUVECs was analyzed by immunoprecipitating VEGF from media using anti-VEGF antibody and probing with anti-PAR antibody after transferring to NC membrane (Figure: 7.11.). The level of PAR modified VEGF was 63% in the case of medium from cells on polylysine whereas it was only 25% in the case of HBD and 29% for FN. This result correlates with the angiogenic potency of the affinity isolated VEGF in CAM assay.

Figure: 7.11. PAR modification of VEGF in cells maintained on HBD:- HUVECs were maintained in culture for 48 hours on polylysine, FN and HBD coated culture dishes. The media were collected, VEGF was immunoprecipitated using anti-VEGF antibody, electrophoresed and immunoblotted using anti-PAR antibody (A). The blot given is of a representative experiment. The intensity of the bands is given as percentage, considering the intensity of band identified by anti-VEGF antibody as total VEGF (B). Polylysine – (P), FN and HBD – (H). Values given are the average of three sets of experiments done in duplicate ± SEM.

*Statistically significant compared to polylysine (p<0.05)
#Statistically significant compared to FN bound VEGF (p<0.05)
7.3.8. Level of NAD\(^+\) in cells maintained on HBD of FN

As NAD\(^+\) is the substrate for PAR modification of VEGF, the level of NAD\(^+\) in cells maintained on HBD of FN was analysed (Figure: 7.12.). The results showed that the level of NAD\(^+\) was significantly low in cells maintained on HBD of FN as compared to polylysine and FN.

![Figure 7.12: NAD\(^+\) levels in cells maintained on HBD of FN: HUVECs were maintained in MCDB 131 medium on polylysine (P), FN and HBD (H) for 48 hours and the level of NAD\(^+\) in the cells were estimated fluorimetrically. The values given as nanomoles of NAD\(^+\) are the average of quadruplicate experiments ± SEM. *Statistically significant compared to P (p<0.05)]](image)

7.3.9. HBD binds biologically more active form of VEGF

Solid phase binding of VEGF to FN and HBD followed by ELISA showed that of the total VEGF bound to FN, about 45% was PAR modified while it was about 31% in the case of HBD (Figure: 7.13A.).

Dot blot assay of VEGF to FN and HBD also showed that VEGF bound to both FN and HBD. Of the total VEGF bound to FN, 40% was PAR modified whereas only 25% of VEGF bound to HBD was PAR modified (Figure: 7.13B.) confirming that HBD binds more amount of less PAR modified, biologically more active form of VEGF.
7.3.10. HBD modulates COX-2 in HUVECs in a PKC dependent manner

As FN was found to influence the activities and expression of COX-2, its expression and activity were analysed in cells maintained on HBD and the involvement of PKC dependent signaling was also examined. The total activity of
COX was found to be high in cells maintained on HBD which decreased by more than 50% on treatment with calphostin C (Figure: 7.14.). The results of RT-PCR analysis showed that the mRNA expression of COX-2 was significantly high in cells maintained on HBD as compared to FN and the inhibition of PKC caused a significant decrease in COX-2 mRNA levels in cells maintained on HBD (Figure: 7.15). But the decrease in the expression in cells maintained on FN was not as significant as that with HBD.

Figure: 7.14. Activity of COX in cells maintained on HBD: Isolated HUVECs were maintained in culture on FN and HBD coated plates with (FN+C, H+C) and without (FN, H) calphostin C for 48 hours in MCDB131 medium in a carbon dioxide incubator. At the end of the experiment, activity of COX was assayed in cell lysates as described in the text and expressed as specific activity (nanomoles of oxygen used/min/mg protein). The values given are the average of quadruplicate experiments ± SEM.

* Statistically significant compared to HBD (p<0.05)
# Statistically significant compared to FN (p<0.05)
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7.1. Changes in the levels of COX-2 mRNA – Effect of HBD:

Isolated HUVECs were maintained in culture on FN and HBD coated plates with (FN+C, H+C) and without (FN, H) calphostin C for 48 hours in MCDB131 medium in a carbon dioxide incubator. Cells were harvested, total RNA isolated and mRNA levels of COX-2 was analyzed by RT-PCR. The PCR products were separated on 1.75% agarose gel and bands visualized by ethidium bromide staining (inset). The gel given is from a representative experiment. Relative intensity of the bands were quantitated by BioRad Quantity One 4.5 software and normalized with that for internal control (GAPDH given in figure: 7.8). Values given are the average of quadruplicate experiments ± SEM.

*Statistically significant compared to HBD (p<0.05)
#Statistically significant compared to FN (p<0.05)

7.4. Discussion

As indicated before, endothelial cells express FN and its receptor, \( \alpha_5 \beta_1 \) integrin, and the data presented in the previous chapters and reported from elsewhere indicate that both FN and \( \alpha_5 \beta_1 \) integrin directly regulate angiogenesis (Kim et al., 2000). Because FN is composed of several functional domains, cells can interact with FN at multiple sites. Results presented above suggest that the HBD of FN by itself can promote angiogenic process. Evidence in support of the angiogenic effect of the HBD of FN are (a) HUVECs maintained on HBD of FN developed capillary like network structures and upregulated biochemical markers of angiogenesis such as E-selectin, CD31 and endothelial specific VEGFR2, (b) In in vivo CAM assay, HBD promoted formation of vascular structures and (c) Aortic rings exposed to HBD showed more sprout formation. The pro-angiogenic effect of HBD observed here is consistent with the earlier report that the heparin binding fragment of FN induced proliferation and migration of human retinal endothelial cells, which was relevant to angiogenesis observed in proliferative diabetic retinopathy (Grant et al., 1998).
In vitro cell attachment assay showed that the HUVECs interact with and adhere to HBD. This is consistent with the earlier reports that the carboxy-terminal 33 kDa HBD of FN is active in promoting the adhesion of many cell types such as neurons from developing chicken (Rogers et al., 1987), herpes simplex virus infected HUVECs (Visser et al., 1989), lymphocytes (Liao et al., 1989) and hepatocytes (Kumar and Sudhakaran, 1992); HBD is also found to promote spreading of murine melanoma and fibrosarcoma cell lines in a RGD-independent manner (McCarthy et al., 1990). Inhibition of the attachment of ECs to HBD by heparin suggests that heparin-like cell surface molecules are involved in mediating the adherence to HBD. The cell surface heparan sulphate proteoglycan (HSPG) syndecan-4 has been implicated as the receptor for the HBD (Couchman and Woods, 1999). Syndecan-4 is the major group of proteoglycans on EC surface that can interact with the cytoskeleton and HBD of matrix proteins. Although cell adhesion to FN is primarily dependent on β1 integrins that interact with the cell-binding domain of FN, cooperative signaling from syndecan-4, as a result of the interaction with the HBD of FN is suggested to be critical in the assembly of focal adhesions and actin stress fibers (Wilcox-Adelman et al., 2002). It appears that the interaction of HBD of FN through the cell surface HSPGs plays an important role in angiogenesis. The signaling pathways activated by VEGFs and FGFs have been suggested to be heparan sulfate–dependent, as evidenced by the failure of cells deficient in heparan sulfate biosynthesis to activate these pathways and the ability of exogenous heparin/HSPG to restore their function (Iozzo and San Antonio, 2001). Moreover, wound healing is shown to be associated with a pronounced increase in syndecan-1 and syndecan-4 expression (Gallo et al., 1996). Syndecan-4 knockout mice demonstrate reduced postnatal angiogenesis that may be related to the impairment of EC migration and impaired skin wound healing is thought to be secondary to defective angiogenesis (Echtermeyer et al., 2001).

As discussed before, among a variety of angiogenesis inducing growth factors, VEGF is a key angiogenic factor that can act in an autocrine manner having survival effects on several cell types including endothelial cells (Brusselmans et al., 2005). VEGF is required for the growth and differentiation of ECs. The mRNA expression of this mitogen was found to be significantly high in cells maintained on HBD. Moreover, the expression of VEGFR2, the specific angiogenic marker in endothelial cells and the primary mediator of cellular effects of VEGF, was also found to be significantly high in cells maintained on HBD as compared to those on polylysine and FN coated surfaces.
The level of secreted VEGF protein was also significantly high in cells maintained on HBD as compared to polylysine and was comparable with the VEGF secreted by cells on FN. These results thus suggest that the pro-angiogenic effect of the HBD of FN is mediated through the upregulation of VEGF and its endothelial cell specific receptor VEGFR2 genes. In this context, the recent observation that the C terminal HBD of FN may amplify the biological effects of VEGF by binding to it and modulating its activity is particularly relevant (Wijelath et al., 2006).

Chick CAM assay showed that the VEGF isolated from medium of cells maintained on HBD was more potent in inducing vascularisation. Besides, the VEGF that is bound to HBD was found to be more active biologically. As discussed earlier, the biological activity of VEGF is reported to be modulated at the transcriptional, post-transcriptional and post-translational levels (Neufeld et al., 1999; Semenza, 2001; Xiong et al., 1998), the most important post-translational modification that influences its angiogenic potency being poly ADP ribosylation (PAR) (Kumar et al., 2007; Xiong et al., 1998). Analysis of the PAR modification of VEGF secreted by cells maintained on various substrata showed that cells on FN and HBD secreted VEGF with less PAR modification as compared to that on polylysine. Moreover, solid phase binding assay and dot blot assay showed that the HBD of FN binds VEGF and of the total VEGF bound, the amount of unmodified VEGF was high as compared to PAR modified VEGF. The HBD bound form of VEGF was biologically more active in inducing vascularisation in the CAM model due to the low level of PAR modification. The association of VEGF and its receptor with FN and \(\alpha_5\beta_1\) integrin respectively is reported to enhance the biological activity of VEGF (Orecchia et al., 2003; Wijelath et al., 2006). Wijelath et al (Wijelath et al., 2006) showed that the association between VEGF and HBD of FN is required for the full effects of VEGF-induced EC migration and proliferation. Our results suggest that this domain of FN can bind to more biologically active form of VEGF which may increase the availability of the biologically potent form of VEGF to the cells.

Treatment of HUVECs maintained on HBD with PKC inhibitor, calphostin C caused a downregulation in the expression of VEGFR2 but not VEGF. Further, the protein levels of VEGF, E-selectin, CD 31 and vWF also decreased significantly on PKC inhibition suggesting the involvement of PKC dependent intracellular signaling pathway in mediating the effect of HBD of FN. Syndecan-4 and PKC have a close
functional association as PKC recruits syndecan-4 to focal adhesion sites (Baciu and Goetinck, 1995) and conversely, ligation of syndecan-4 through phosphatidyl inositol phosphate potentiates PKC activity (Horowitz et al., 1999). The mitogenic effect of VEGF is also shown to decrease significantly by PKC inhibition in HUVECs (Wellner et al., 1999).

In addition to influencing the VEGF activity and angiogenic marker levels, HBD was found to influence COX-2, an EC function relevant to angiogenesis. As discussed in previous chapters, COX-2 and its product prostaglandins are important modulators of angiogenesis. The upregulation of COX-2 expression and activity of COX indicate that one of the mechanisms whereby HBD influences the angiogenic phenotype of ECs is by the production of pro-angiogenic prostaglandins such as PG\(_E_2\). Moreover, as with the angiogenic markers, the inhibition of PKC caused a significant reduction in the COX activity in cells maintained on HBD of FN.

Thus it appears that HBD of FN exerts its effect by interacting with cell surface HSPG, syndecan-4 and thereby activating an intracellular signaling pathway involving PKC (Figure: 7.16.). Decreased level of \( \text{NAD}^+ \), in cells maintained on HBD of FN leads to decreased poly ADP ribosylation of VEGF, rendering it biologically more active. The binding of less PAR modified VEGF to the HBD of FN increases the availability of biologically more active form of VEGF to the cells. Further, it appears that PKC mediated downstream signaling pathway causes an upregulation of COX-2 gene and thereby COX activity.

FN fragments have been found to have activities not found in the intact molecule with greater or different biological activity than the parent protein. Some selected FN fragments have been shown to affect proliferation, promote the adhesion, spreading and migration of vascular endothelial cells (Grant et al., 1998; Huebsch et al., 1995). Degradation of FN by proteases occurs near cells undergoing neoplastic transformation, diabetic retinopathy and arthritis and FN fragments have been identified at sites of inflammation, injury and destruction by metastatic tumor cells (Wilson et al., 2003; Xie et al., 1993). Our results suggest that the HBD, a proteolytic fragment of FN accumulating in these conditions may contribute to the neovascularisation associated with these diseases by modulating the biological potency of growth factors and production of soluble angiogenic modulators.
Figure 7.16. Scheme for mechanism of angiogenic nature of HBD: - HBD of FN interacts with cell surface HSPG, syndecan and activates PKC dependent intracellular signaling pathway that may involve activation of transcription factors such as NF-κB. Decreased level of NAD in cells maintained on HBD leads to decreased poly ADP ribosylation of VEGF, increasing its biological potency and increases its binding to the HBD of FN thereby increasing the availability of biologically more active form of VEGF to the cells. PKC also leads to increased activity and expression of COX.