Imbalance of Angiogenesis in Diabetic Complications: The Mechanisms

Zoya Tahergorabi, Majid Khazaei

Department of Physiology, Isfahan University of Medical Sciences, Isfahan, Iran

Correspondence to:
Asso. Prof. Majid Khazaei,
Department of Physiology, Faculty of Medicine and Child Growth and Development Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail: khazaei@med.mui.ac.ir

Date of Submission: May 06, 2012

Date of Acceptance: Oct 07, 2012

How to cite this article: Tahergorabi Z, Khazaei M. Imbalance of angiogenesis in diabetic complications: The mechanisms. Int J Prev Med 2012;3:827-38

ABSTRACT

Type 2 diabetes mellitus is a complex disease and a chronic health-care problem. Nowadays, because of alteration of lifestyle such as lack of exercise, intake of high fat diet subsequently obesity and aging population, the prevalence of diabetes mellitus is increasing quickly in around the world. The international diabetes federation estimated in 2008, that 246 million adults in worldwide suffered from diabetes mellitus and the prevalence of disease is expected to reach to 380 million by 2025. Although, mainly in management of diabetes focused on hyperglycemia, however, it is documented that abnormalities of angiogenesis may contribute in the pathogenesis of diabetes complications. Angiogenesis is the generation of new blood vessels from pre-existing ones. Normal angiogenesis depends on the intricate balance between angiogenic factors (such as VEGF, FGF_2, TGF-β, angiopoietins) and angiostatic factors (angiostatin, endostatin, thrombospondins). Vascular abnormalities in different tissues including retina and kidney can play a role in pathogenesis of micro-vascular complications of diabetes; also vascular impairment contributes in macrovascular complications e.g., diabetic neuropathy and impaired formation of coronary collaterals. Therefore, identifying of different mechanisms of the diabetic complications can give us an opportunity to prevent and/or treat the following complications and improves quality of life for patients and society. In this review, we studied the mechanisms of angiogenesis in micro-vascular and macro-vascular complications of diabetes mellitus.

Key words: Angiogenesis, diabetes mellitus, diabetic complications, macro-vascular, micro-vascular

Review Article

INTRODUCTION

Type 2 diabetes mellitus is a chronic condition and considered as a major cause of mortality and morbidity for its micro-vascular (such as retinopathy, nephropathy and neuropathy) and macro-vascular (coronary heart disease, peripheral vascular disease and stroke) complications.[1-3] Diabetes was previously considered as the eighth cause of death around the world but now is the fifth cause of death following infections, cardiovascular disease, cancer and trauma.[4] Type 2 diabetes is founded secondary to

insulin resistance and hyperinsulinemia condition.[5] It is a multi-factorial disease that in its development in addition to genetic predisposition, play role a number of environmental factors such as poor nutrition, lack of physical activity and obesity.[6] Among these factors, obesity has more potent relationship with type 2 diabetes.[7] Nowadays, type 2 diabetes mellitus is founded as epidemic.

It is evaluated that 285 million persons presently have diabetes and it is estimated it will increase to 438 million that is equivalent with 7.8% of the world adult population by 2030. Improvement in national socio-economic status of human societies that is indicated with increase in obesity and diabetes population can correlate with increasing in the incidence of diabetes.[8]

Angiogenesis is the generation of new blood vessels from pre-existing ones that provides sufficient blood flow and oxygen for growing tissues. Endothelial cells are the main cells involved in the angiogenesis process. Physiological angiogenesis contributes in events such as pregnancy, embryonic development, wound healing and menstruation,[9] however, disturbances in physiologic angiogenesis can participates in various human diseases including cancer, cardiovascular diseases, diabetic complications, ocular disease and chronic inflammation[10] in the form impairment angiogenesis that leads to diabetic vasculopathy[11,12] or excessive angiogenesis that can cause to diabetic retinopathy and nephropathy and inhibited angiogenesis in transplant rejection in diabetic recipients.[13]

Angiogenesis is consisting of several regulated stages. The first is proteolysis of the ECM (extracellular matrix) through proteolytic systems; for example plasminogen/plasmin and MMP (matrix metalloproteinase) systems. Then, migration of endothelial cells toward an angiogenic stimulus, consequently proliferation of the endothelial cells to supply of sufficient number of cells and, finally, tube formation into a patent three dimensionally tubular structure and maturation blood vessel with lumen formation. Normal angiogenesis depends on the intricate balance between angiogenic (e.g., VEGF: Vascular endothelial growth factor, FGF2: Fibroblast growth factor), TGF-β: Transforming growth factor and angiopoietin) and antiangiogenic factors (angiostatin, endostatin, thrombospondins).[14]

Insufficient angiogenesis with limiting entrance of inflammatory cells and poor supply of oxygen and nutrients in wound space has an important role in the impaired wound healing process. In one experiment, topical administration of high glucose to wounds of non-diabetic rats leads to inhibition of the normal angiogenesis that shows direct role of high glucose levels in decreased angiogenesis in diabetes.[15] In a study on twenty patients that placed undergo coronary artery bypass surgery, increased VEGF expression in the myocardium of diabetic patients in comparison with non-diabetic patients and decreased expression levels of VEGF receptor 1 and 2 that shows relationship of diabetic cardiovascular complication with dysregulation of neovascularization[16] serious complications of diabetes have been demonstrated in Figure 1.

Angiogenesis in diabetic retinopathy

Diabetic retinopathy begins with micro-aneurysms. Then, it develops into exudative changes (leakage of lipoproteins and blood) that lead to macular edema, ischemic changes (infarcts of nerve-fiber layer), collateralization (intraretinal microvascular abnormalities) and dilatation of venules. Finally, the proliferative changes (abnormal vessels on the optic disk and retina, proliferation of fibroblasts and vitreous hemorrhage) occur.[18]

In recent years, following improved control of glycemia status, blood pressure and lipid levels in persons lead to remarkable decrease in the

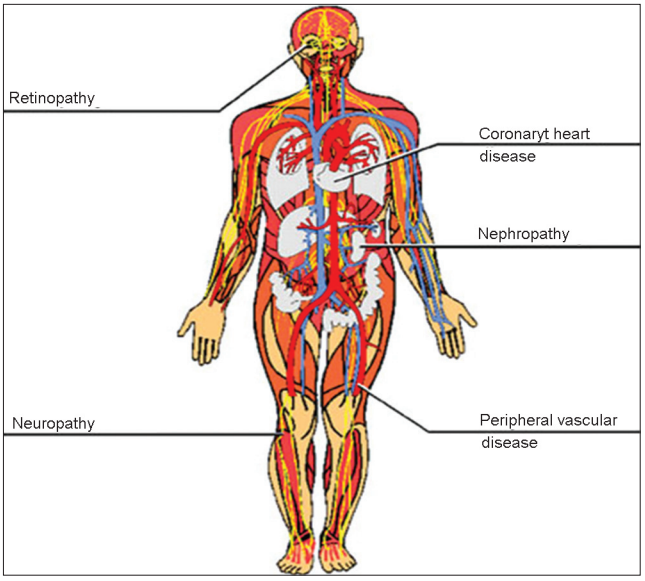

Figure 1: Serious complications of diabetes[17]

prevalence and incidence of diabetic retinopathy and vision impairment.[19] One population-based epidemiological study has been shown that the estimated annual incidence of proliferative diabetic retinopathy and vision impairment from 1980 to 2007 decreased by 77% and 57% in diabetic patients Type 1 respectively.[20]

Close relationships in the form of physical and biochemical among neurons, glial cells and distinct vasculature in the central nervous system is termed neurovascular unit that can retina also is considered as a neurovascular unit.[21,22] It consists of close proximity of micro-vasculature segments with astrocytes, muller cells, amacrine and ganglion neurons that supplies oxygen and nutrients.[23] Macular edema in the non-proliferative phase of diabetic retinopathy is associated with occlusion and leakage of retinal vessels and in the proliferative phase there are angiogenesis and cluster of high permeable vessels.[24] Macular edema closely associated with thickening of the central fovea and vision loss.

Effective treatment with anti-VEGF agents such as ranibizumab and bevacizumab shows that VEGF through several mechanisms involve in pathogenesis of diabetic macular edema.[25,26] The first mechanism is through hypoxia and AGE_S (advanced glycosylation end products) that AGE_S correlate with prolonged hyperglycemia and produce ROS (reactive oxygen species) and ROS increases VEGF expression. Hypoxia also induces HIF-1α (hypoxia inducible factor), which stimulates production of VEGF and VEGF receptors. VEGF leads to mitogenic effect and proteolysis of basement membrane that is the first stage of angiogenic process and cause to proliferative diabetic retinopathy.[27] AGE_S are biochemical end products of non-enzymatic glycosylation in diabetic patients with poor glycemic control. AGE_S lead to ECM expansion in phenomenon of diabetic tissue fibrosis and irreversible biochemical changes in protein structure and function.[13]

The second mechanism is the activation of PKC (protein kinase C) isoforms especially protein kinase Cβ that leads to VEGF-induced vascular penetrability. Protein kinase Cβ with targeting of tight-junction protein, occludin, causes to ubiquitin-mediated endocytosis of tight junction and increases vascular permeability.[28,29] Several clinical trials have demonstrated that oral administration of ruboxistaurin by inhibiting protein kinase Cβ reduced number of patients with sustained moderate visual in comparison with placebo group.[30]

Extracellular proteases of UP-A okinase plasminogen activator) and MMP-2 and MMP-9 also involve in retinal vascular permeability with degradation of tight junction proteins.[31] Also, lack of proper function of pericytes may contribute in vascular permeability. Deletion of PDGFβ (platelet derived growth factor) gene participates in formation of walls of newly formed vessels with recruiting of pericytes and creates a phenotype similar to diabetic retinopathy with increased angiogenesis and vascular damage.[32] Gerald *et al*. recently showed that Src-homology 2 domain containing tyrosine phosphatase 1 can inhibit PDGF signaling through the Akt survival pathway. This tyrosine phosphatase up regulated by expression of protein kinase Cδ that is induced by high glucose levels. Therefore, inhibition of PDGF signaling can lead to pericyte cell death, vascular damage and diabetic retinopathy.[33]

Obesity and diabetes are associated with systemic inflammation.[34] There are excess releases of retinal inflammatory mediators such as IL-1β (interleukin-1ß), TNF-α (tumor necrosis factor), ICAM-1 (intercellular adhesion molecule) and Ang-II (angiotensin) in diabetic persons that can contribute in VEGF permeability.[35] IL-1β and TNF-α aggregate in the vitreous and macrophage migration into neurosensory retina is created in proliferative diabetic retinopathy.[21,36] Therefore, glucocorticoids such as fluocinolone decrease retinal inflammation and increase tight junction protein expression which may improve the integrity of the blood retina barrier (is including glial-cell, pericyte and neural interactions for control the flow of fluids and blood metabolites into neural parenchyma) can be considered as treatment for diabetic retinopathy.[37]

Activation of the polyol metabolic pathway also participates in diabetic retinopathy. During this, glucose is reduced to sorbitol through the enzyme aldose reductase. Sorbitol accumulation leads to decrease in myo-inositol content, abnormal phosphoinositide metabolism and reduced Na^+-K+ATPase activity that can increase in collagen cross-linking and vascular permeability. Vascular permeability leads to extravasations of proteinases and thus accelerates

neovascularization.[13] A depiction of mechanisms of angiogenesis in diabetic retinopathy has been shown in Figure 2.

Angiogenesis in diabetic nephropathy

Glomerular hypertrophy, as one of the outcomes of diabetic nephropathy in both animals and humans is characterized with increased length and number of capillaries. It is demonstrated that 10 and 50 days after injection of streptozotocin to rats and induction of diabetes, the average total surface area, length and numbers of glomerular capillaries increased in diabetic rats in comparison with controls.[38] Abnormal surplus vessels in diabetic nephropathy that are structurally immature lead to an increase in vascular permeability that in turn cause to extravasations of plasma proteins.[39] In type 2 diabetes, glomerular lesions are created in the form of increase in glomerular endothelial cell number in consequence of imbalance in cell proliferation and apoptosis. Main role in this process is attributed to VEGF-A expression following high glucose levels in the early phases of diabetes.[40] Of course, high glucose levels by stimulating of transcription of TGF-β in vascular smooth muscle cells alone can cause to endothelial cell proliferation.[41] Furthermore, overexpression of TGF-β1 and TGF-β2 receptors besides VEGF in renal cortical and glomerular tissue of both human and animal models of diabetic nephropathy also involved. Therefore, hyperglycemia indirectly can induce VEGF overexpression mediated by TGF-β.[13]

Another mechanism in the abnormal angiogenesis is glomerular hypertension. In a study, it has been shown that treatment of hypertension in diabetic patients with ACE (angiotensin converting enzyme) inhibitors or β-blockers for 8 years suppress development glomerular lesions and extra vessel formation.[42] Furthermore, effective reduction of blood pressure by VEGF-A inhibition has been showed. Vessels as a by-pass to decrease in intra-glomerular pressure make abnormal vessels connection between intra-glomerular capillaries to peritubular capillaries. Thus, decrease of systemic blood pressure by reduction of intra-glomerular pressure might decrease development of by-pass vessels.[39]

Abnormal angiogenesis in diabetes is mediated through VEGF-A family especially VEGF-A_{164} and VEGF-A188 isoforms and VEGFR1 and VEGFR2 receptors.[43,44] High levels of VEGF-A also lead to vascular permeability in the glomeruli,[45] Physiological levels of NO (nitric oxide) creates low vascular permeability but NO levels too high or too low can lead to hyper-permeability that

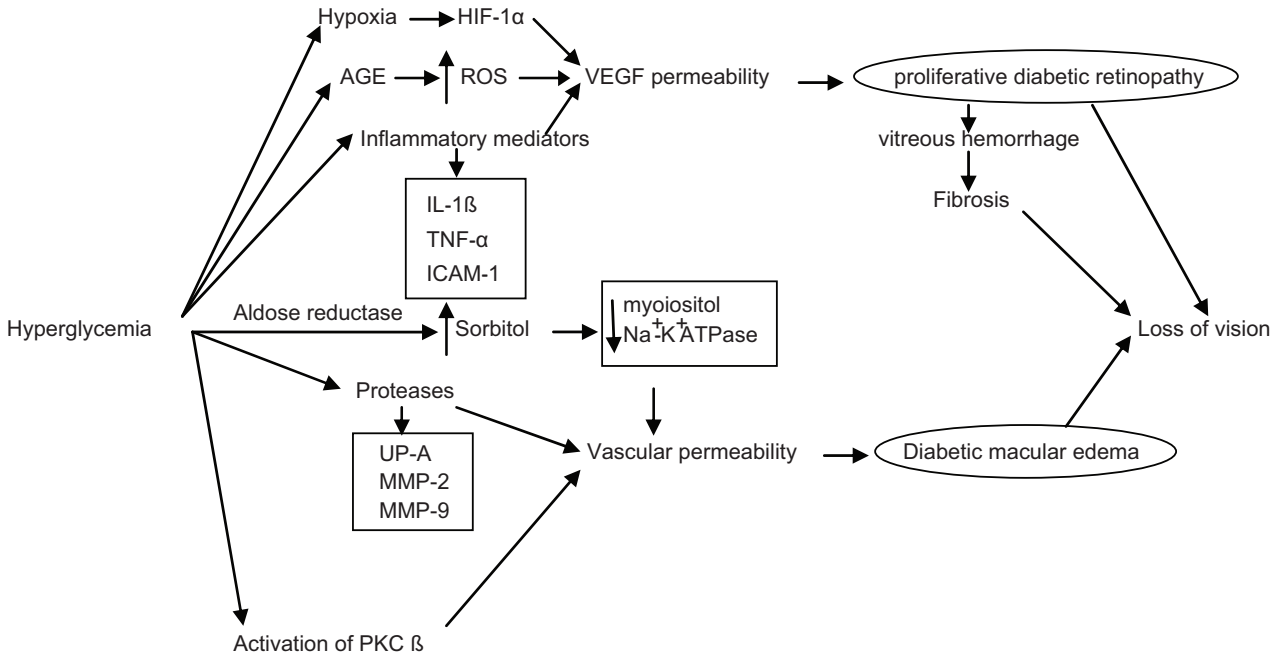

Figure 2: Mechanisms of angiogenesis in diabetic retinopathy

in turn lead to glomerular injury and diabetic nephropathy.[39]

The primary origin of VEGF-A in the kidney are podocytes and tubular epithelial cells that podocytes are as main regulators of macro-molecular permeability. Studies have been demonstrated that up regulation of VEGF-A in early stages of diabetic nephropathy is created for initial development of disease, While, decrease in VEGF-A in the later stage can be founded following of interruption of podocytes and tubular cells in advanced phases of cellular damage.[46] Angiopoietins are another family of growth factors in the development of diabetic nephropathy. Angiopoietin 1, 2 are ligands for Tie-2 receptor tyrosine kinase and angiopoietin 2 is a natural antagonist of angiopoietin-1.[47] Changes in the expression of angiopoietins in the form of reduced ratio of angiopoietin 1 to angiopoietin 2 beside VEGF-A can be involved in the development of diabetic nephropathy.[39]

De Vriese's group has demonstrated that treatment of diabetic rats induced by streptozotocin with a monoclonal anti-VEGFA antibody decreased hyper-filtration, albuminuria and glomerular hypertrophy. Of course, should be cautious in using VEGF-A inhibitors in the kidney disease. For example, although there are useful effects of anti-VEGF antibody in animal models of diabetes but since endothelial cells need to VEGF-A in physiological conditions, VEGF-A inhibition can cause to endothelial injury.[48]

Angiogenesis in diabetic neuropathy

The peripheral nerves system in diabetic patients is impaired in the form loss of sensation and reduced awareness of injury and developing skin ulcers more commonly in the lower extremities.[13] The prevalence of diabetic neuropathy is 7% in the first year of diagnosis and 50% for patients more than 25 years of diagnosis.[49]

Pathogenic mechanisms of diabetic neuropathy including: Non-enzymatic glycation of proteins involved in neural function, changes in neural polyol metabolism, prevention of production of angiogenic and neurotrophic growth factors following hyperglycemia, production of ROS and micro-vascular disease with impaired blood circulation in diabetic nerves.[50]

Structural abnormalities are in the epineurial vessels consist of: Arteriolar attenuation, venous distension, arteriovenous shunting, new vessel formation with intimal hyperplasia and hypertrophy and denervation. Micro-angipathic alterations in endoneurial vessels are in diabetic patients including: Hyalin thickening and endothelial hypertrophy, hyperplasia and Basement membrane thickening and pericyte loss. Increased blood vessel number in diabetic nerves in early stages of diabetes mellitus is created while decreased blood vessel number in chronic diabetic condition occur due to ischemia and impaired neovascularization respectively.[51]

Hyperglycemia through non-enzymatic and enzymatic mechanisms leads to oxidative stress and increased production of ROS. ROS promotes peroxidation of lipid membranes, proteins and DNA that in turn affect on cell structure and function.[52] Up regulation of the vascular RAS (renin-angiotensin system) and elevation of endothelin-1 levels have been demonstrated due to oxidative stress in diabetic rats that both may lead to decreased peripheral nerve blood flow in diabetes. In agreement with data, endoneural hypoxia that leads to impaired blood flow in diabetic rats is improved by AT1 receptor antagonists.[53] In animals with diabetic neuropathy has been shown decrease in growth factors with both angiogenic and neurotrophic function due to oxidative stress. These growth factors direct blood vessels and nerves to their tissue targets.[54] In some studies, administration of VEGF-C and VEGF-A and neurotrophic factors e.g., NGF (nerve growth factor), IGF1 (insulin like growth factor) and IGF2 through improvement of micro-circulation in damaged nerves in animal models has been demonstrated.[55] Many factors have dual effects angiogenic and neurotrophic that are referred to as Angioneurin including, VEGF, IGF1, NGF, and FGF2. VEGF in addition to angiogenic effects directly involve in the growth, survival and protection of neural and axonal outgrowth. NGF also shows neurotrophic and neurotropic effects and leads to maintenance, survival in neural cells. Furthermore IGF_s drive growth and differentiation of neurons. Therefore, disturbances in metabolism and insufficient vascular support and neurotrophic factors mainly contribute in pathogenesis of diabetic neuropathy.[56] On the other hand, impaired release of endothelium-dependent relaxing factor has been shown in the aorta of diabetic rats.[53] Also, exposure to high glucose levels and decreased anti-oxidants e.g.. glutathione promotes

activation protein kinase C pathway. PKC inhibits Na^+-K+ATPase and decreases production of eNOS. eNOS reduction and increased PAI-1 (plasminogen activator inhibitor-1) lead to blood flow disturbances and vascular obstruction respectively. Thus PKC pathway contributes in the pathogenesis of diabetic microcirculation complication.[56,57] As a matter of fact, oxidative stress makes interrelation in many glucose-related pathogenesis of diabetic neuropathy. In the study, it has been shown that injection of $BMNC_S$ (bone marrow derived mononuclear cells) intramuscular along the sciatic nerve with angiogenic and/or neurotrophic factors can be considered useful treatment. Injection of $BMNC_S$ improves of vascularity of diabetic nerves with stimulation of neovascularization through EPC_S (endothelial progenitor cells) and MSC_S (marrow stromal cells) and restore of NCV (nerve conduction velocity) to near normal value in diabetic neuropathy.[51] Graphical representation of mechanisms of angiogenesis in diabetic neuropathy has been shown in Figure 3.

Impaired wound healing in diabetes

The normal wound healing process is containing 5 stages: Hemostasis, inflammation and debridement, proliferation, epithelialization and remodeling. In non-healing, diabetic wound is observed disturbances in two stages: The inflammation/debridement and the proliferation stages.[13]

In skin-wound models that is created by incision on the back of db/db mice, impairment of wound healing is appeared with decreased angiogenesis, delayed formation of granulation tissue, reduced collagen content, decreased arteriolar number and density, loss of vasculature tone and decrease in the cross-sectional area of new vessel walls.[58] Chronic non-healing ulcers in diabetic patients localized on pressure points of the foot, including: The metatarsophalangeal joints, ankles and heels.[13] Diabetic foot is not a typical complication of the late stages of diabetes, but also occurs in patients with newly diagnosed diabetes. Diabetic foot ulceration forms 84% of all diabetes-related lower leg amputations.[59,60]

Angiogenesis is a necessary component of the wound healing process. In diabetic patients, there is insufficient angiogenesis in wound site in the form decreased endothelial cell proliferation and decreased cell and growth factor response.[13] For example in response to hypoxia there is increased threefold wild type fibroblast production of VEGF whereas, is not up regulated diabetic fibroblast production of VEGF in hypoxia status.[61] Also, in the presence of glucose, down regulation of VEGFR3, VEGF-C and VEGF-D[62] leads to impaired angiogenesis, EPC dysfunction, fewer platelets, reduced granulation formation and defective lymphatic vasculature formation.[63]

Furthermore, impaired wound healing following decreased production of NO via disruption of eNOS phosphorylation has been demonstrated. Abundant ROS production is other mechanism in delayed wound healing like, anti-oxidants such as vitamin

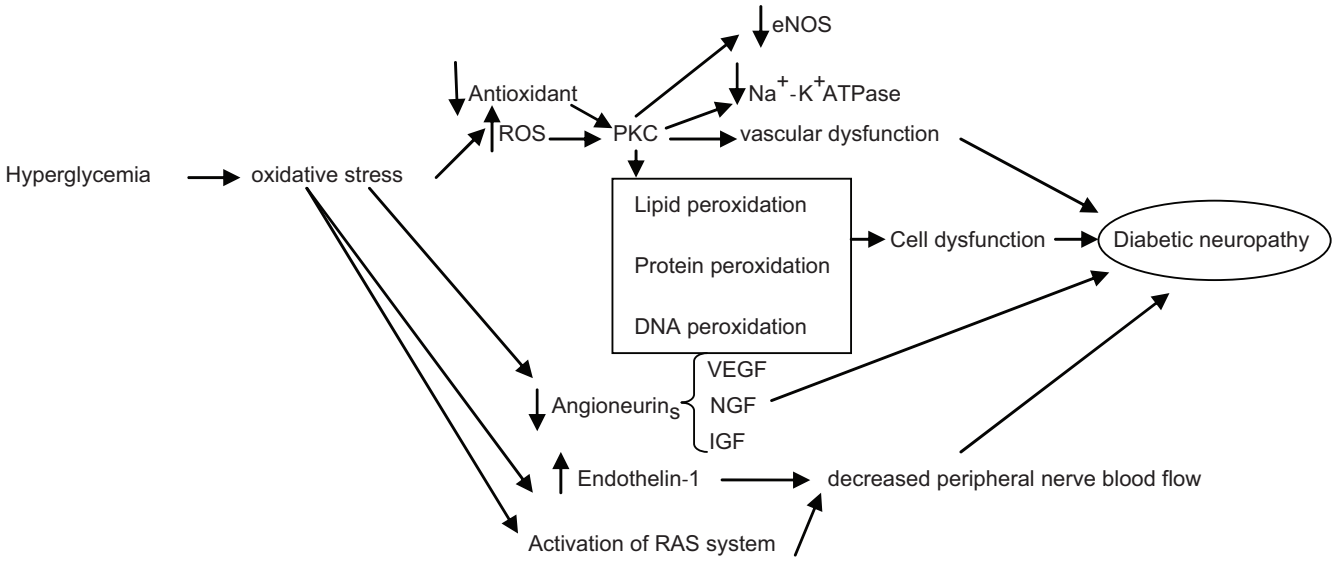

Figure 3: Mechanisms of angiogenesis in diabetic neuropathy

E have been reported are effective in acceleration of diabetic wound healing and angiogenic response.[64]

Diabetic patients with critical limb ischemia benefit from intramuscular VEGF plasmid DNA and would have decreased amputation.[65] Nowadays for overcome wound healing problem in diabetes, nanotechnology has extensive applications in medicine including: Use of silver nanoparticle in cleaning the wound infection.[66] Enhanced wound closing and increase of collagen synthesis by curcumin nanoparticle.[67]

Malignancy and diabetes

Epidemiological studies show that approximately 5% of new cancer cases directly related to overweight.[68] Based on meta-analysis of several studies, type 2 diabetes in comparison with type 1 more commonly is associated with development of some cancers.[69] For instance, relative risk of liver, pancreatic and endometrial cancers with type 2 diabetes is greater than two fold, while relative risk for colorectal, breast and bladder cancer is slightly lower (1.2-1.5) fold. Relative risk of lung cancer is less than 1 and for other cancers (e.g., kidney, non-Hodgkin lymphoma) evidence is doubtful.[70]

Possible mechanisms for relationship between cancer and diabetes type 2 are including: Hyperinsulinemia, hyperglycemia, inflammation and adipokine secretion disturbances. These mentioned mechanisms are created following dysfunction of adipose visceral tissue. Experimental and epidemiological evidences show relationship between diabetes type 2 and cancer that is more consistent with the hyperinsulinemia compared with hyperglycemia. Insulin with mitogenic properties and expression of insulin receptors by cancer cells and increased levels of insulin like growth factor-1 (IGF-1) promote tumor cell growth in type 2 diabetes. Hyperglycemia could be a confounder in the increased risk of cancer in patients with type 2 diabetes.[71,72]

Once insulin interacts with its receptor, it is activated several signaling pathways that lead to increase of proliferation and decrease of apoptosis.[71] Hyperinsulinemia also indirectly with reduction of hepatic production of insulin like growth factor binding protein (IGFBP-1) and IGFBP-2 leads to increase of free active IGF-1 that act as a growth factor in cancer cells.[73] Fuels requirement of tumor growth can is supplied through glycolysis with generation ATP. Furthermore, hyperglycemia has mitogenic activity.[74]

Obesity is associated with low grade systemic inflammation that during it pro-inflammatory factors such as CRP (C-reactive protein), IL-6 (Interleukin-6), and TNF-α increased[75,76] and anti-inflammatory factor of adiponectin decreased. Inflammatory and stromal cells of adipose tissue in turn produce angiogenic factors such as VEGF. Excess production of pro-inflammatory adipokines by lipocytes through activation of NF-κB signaling pathway and increase of oxidative stress contribute in obesity-induced carcinogenesis.[77] Activation of these signaling pathways also leads to inhibition of apoptosis and development of insulin resistance. Adiponectin under normal condition is insulin sensitizer and proapoptotic adipokine that in obesity there is decrease secretion of adiponectin.[78]

On the other hand, PAI-1 a serine protease inhibitor is produced in visceral adipose tissue. Overexpression of PAI-1 associated with many obesity related cancers, particularly colorectal cancer.[79] PAI-1 acts indirectly through activation of MMP that play role in extracellular matrix remodeling. Intensity of colorectal cancer has been demonstrated that associated with high levels of (MMP-2, MMP-7, MMP-9 and MMP-13).[80-82] Serum and tissue MMP-9 increased in pancreatic cancer in comparison with healthy controls.[83] Therefore, PAI-1 through changes of the ECM cause to growth factor secretion, angiogenesis and loss of cell adhesion contributes in local growth cancer and cell migration.[84]

Several evidences show relationship between leptin level and colorectal cancer risk.[85,86] Leptin resistance (high leptin levels) there is in some obese patients.[87] Leptin acts through mitogenic and anti-apoptotic effects in addition to up regulation VEGF mRNA expression that can be reduced by MAP (mitogen activated protein) kinase and PI3-K (phosphatidylinositol 3-kinase) inhibitors.[88]

In a study of 120 patients that suffered from metastatic colon cancer, time progression of cancer is studied. Patients are placed in two groups, according with type of treatment. 40 patients received chemotherapy only and 80 patient bevacizumab (anti-angiogenic agent) as well. In the bevacizumab group, there was shorter time to progression in high visceral fat patients

in comparison with low visceral fat patients, but in chemotherapy, group was observed on difference basis on level of visceral fat.[89] This result can be attributed to several angiogenic factors that are expressed highly in obese adipose tissue and decrease effectiveness of anti-angiogenic therapy.[90]

Diabetes and cardiovascular diseases

Cardiovascular disease (CVD) leads to 65% of deaths in diabetic patients. One of the macro-vascular complications of diabetes is coronary artery disease (CAD).[91] In one population-based study was cleared the 7-year incidence of first MI (myocardial infarction) or death in diabetic patients was 20% against only 3.5% in nondiabetic patients. Rate of recurrence MI also increased in diabetic patients.[92]

Diabetes creates alterations in function of some cell types, including endothelial cells, Smooth muscle cells and platelets are termed vascular dysfunction. Also insulin resistance, hyperglycemia and dyslipidemia can cause to arterial atherosclerosis in diabetic patients.[91]

Hyperglycemia inhibits production of NO in endothelial and vascular smooth muscle cells by blocking eNOS and increase of oxidative stress and generation of ROS especially superoxide anion (O^{2-}). Also there is in type 2 diabetes reduction of endothelium derived NO.[93,94] High glucose levels in addition to reduced eNOS cause to iNOS overexpression[95] that both can inhibit HIF-1α activity (HIF-1α is one of the key genes up regulated by hypoxia and it mediate VEGF gene transcription).[96] NO is downstream mediator of HIF-1α stabilization and a series angiogenic factors such as bFGF, TGF-β and angiopoietin-1. NO stimulates angiogenesis in addition to some steps process of angiogenesis including vasodilatation-induced increase in local blood flow, endothelial proliferation and migration function.[97] Therefore, impaired NO production and overexpression of iNOS with increasing free radical O^{2-} production can inactivate NO, lead to impaired myocardial angiogenesis and inadequate development collateral blood vessels in diabetic patients with coronary artery disease.[98]

Furthermore, production of vasoconstrictors especially endothelin-1 that interacts with endothelin-A receptor on vascular smooth muscle cell increases in diabetes. Endothelin-1 also stimulates RAS system that leads to vascular smooth muscle hypertrophy.[91] Diabetes with advance production of MMP_S leads to degrade of collagen that in turn creates mechanical stability in the plaques fibrous cap. Therefore, collagen breakdown can make plaques susceptible to rupture and thus trigger thrombus formation.[99]

Activation of PKC, following hyperglycemia in vascular smooth muscle cells, endothelial cells and platelets stimulates production of free radical ($^{2-}$). Insulin resistance also with releasing of free fatty acids from adipose tissue produces PKC.[100] Expression of VEGF (a high specific growth factor for endothelial cells *in vivo* and *in vitro* conditions) increases excessively after MI in non-diabetic patients, thereby contributes in formation of collateral vessels in coronary atherosclerosis, but there is insufficient collateral vascular formation in diabetic patients.[101] In some microvascular tissues in diabetes there is increased VEGF expression in consequence hyperglycemia, AGE and oxidative stress that creates pathologic angiogenic response, however, response of myocardium in diabetic patients is different.[101] It has been demonstrated that VEGF mRNA, its protein and receptors all remarkably decrease in short term experimental both diabetic rats and humans that leads to death following MI[91,94,102] against in another study has been shown VEGF mRNA transcript increased in long term (3 months) in consequence long term hypoxic stress and up regulation of the myocardial RAS system in heart of experimental diabetic rats. But the mRNA expression of flt-1 and flk-1 receptors decreased through reduced Akt phosphorylation and eNOS protein expression and phosphorylation that are important signal pathways in endothelial cell proliferation, migration and survival.[98,103-106] Down regulation of the VEGF receptors in the long term can be involved in aggravation of ischemic state in diabetic patients.[101]

CONCLUSIONS

Diabetes is an increasing public health problem as a result of changes in life style such as high fat diet and consequently obesity, physical inactivity in worldwide. The new term of diabesity is used due to the close association between obesity and type 2 diabetes. Identifying different mechanisms of the diabetic complications including angiogenesis can

be helpful for prevention and/or management of complications and thus reduce the rate of complications, as well as large economic impact of disease on the patients and society.

REFERENCES

1. Golden SH. Emerging therapeutic approaches for the management of diabetes mellitus and macrovascular complications. Am J Cardiol 2011;108:59B-67.
2. Edwards MS, Wilson DB, Craven TE, Stafford J, Fried LF, Wong TY, *et al*. Associations between retinal microvascular abnormalities and declining renal function in the elderly population: The Cardiovascular Health Study. Am J Kidney Dis 2005;46:214-24.
3. Jeerakathil T, Johnson JA, Simpson SH, Majumdar SR. Short-term risk for stroke is doubled in persons with newly treated type 2 diabetes compared with persons without diabetes: A population-based cohort study. Stroke 2007;38:1739-43.
4. Roglic G, Unwin N, Bennett PH, Mathers C, Tuomilehto J, Nag S, *et al*. The burden of mortality attributable to diabetes: Realistic estimates for the year 2000. Diabetes Care 2005;28:2130-5.
5. Dixon JB. Obesity and diabetes: The impact of bariatric surgery on type-2 diabetes. World J Surg 2009;33:2014-21.
6. Li S, Zhao JH, Luan J, Langenberg C, Luben RN, Khaw KT, *et al*. Genetic predisposition to obesity leads to increased risk of type 2 diabetes. Diabetologia 2011;54:776-82.
7. Leibson CL, Williamson DF, Melton LJ 3rd, Palumbo PJ, Smith SA, Ransom JE, *et al*. Temporal trends in BMI among adults with diabetes. Diabetes Care 2001;24:1584-9.
8. IDF. IDF diabetes atlas. 4th ed. Brussels (Belguim): International Diabetes Federation; 2009.
9. Twigg SM, Chen MM, Joly AH, Chakrapani SD, Tsubaki J, Kim HS, *et al*. Advanced glycosylation end products up-regulate connective tissue growth factor (insulin-like growth factor-binding protein-related protein 2) in human fibroblasts: A potential mechanism for expansion of extracellular matrix in diabetes mellitus. Endocrinology 2001;142:1760-9.
10. Xue Y, Lim S, Brakenhielm E, Cao Y. Adipose angiogenesis: Quantitative methods to study microvessel growth, regression and remodeling *in vivo*. Nat Protoc 2010;5:912-20.
11. Khazaei M, Salehi E, Rashidi B. Pan-PPAR Agonist, Bezafibrate, Restores Angiogenesis in Hindlimb Ischemia in Normal and Diabetic Rats. Int J Pept 2012;2012:637212.
12. Khazaei M, Salehi E, Rashidi B, Javanmard SH, Fallahzadeh AR. Role of peroxisome proliferator-activated receptor beta agonist on angiogenesis in hindlimb ischemic diabetic rats. J Diabetes Complications 2012;26:137-40.
13. Martin A, Komada MR, Sane DC. Abnormal angiogenesis in diabetes mellitus. Med Res Rev 2003;23:117-45.
14. Goodwin AM. *In vitro* assays of angiogenesis for assessment of angiogenic and anti-angiogenic agents. Microvasc Res 2007;74:172-83.
15. Brem H, Jacobs T, Vileikyte L, Weinberger S, Gibber M, Gill K, *et al*. Wound-healing protocols for diabetic foot and pressure ulcers. Surg Technol Int 2003;11:85-92.
16. Sasso FC, Torella D, Carbonara O, Ellison GM, Torella M, Scardone M, *et al*. Increased vascular endothelial growth factor expression but impaired vascular endothelial growth factor receptor signaling in the myocardium of type 2 diabetic patients with chronic coronary heart disease. J Am Coll Cardiol 2005;46:827-34.
17. Available from: http://www.roche.com/pages/facets/3/diabetes_2.jpg. [Last accessed on 2012 Oct 24].
18. Antonetti DA, Klein R, Gardner TW. Diabetic retinopathy. N Engl J Med 2012;366:1227-39.
19. Klein R, Klein BE. Are individuals with diabetes seeing better?: A long-term epidemiological perspective. Diabetes 2010;59:1853-60.
20. Klein R, Lee KE, Gangnon RE, Klein BE. The 25-year incidence of visual impairment in type 1 diabetes mellitus the wisconsin epidemiologic study of diabetic retinopathy. Ophthalmology 2010;117:63-70.
21. Su EJ, Fredriksson L, Schielke GP, Eriksson U, Lawrence DA. Tissue plasminogen activator-mediated PDGF signaling and neurovascular coupling in stroke. J Thromb Haemost 2009;7:155-8.
22. Hawkins BT, Davis TP. The blood-brain barrier/neurovascular unit in health and disease. Pharmacol Rev 2005;57:173-85.
23. Pournaras CJ, Rungger-Brandle E, Riva CE, Hardarson SH, Stefansson E. Regulation of retinal blood flow in health and disease. Prog Retin Eye Res 2008;27:284-330.
24. Gardner TW, Larsen M, Girach A, Zhi X. Diabetic macular oedema and visual loss: Relationship to location, severity and duration. Acta Ophthalmol 2009;87:709-13.
25. Bai Y, Ma JX, Guo J, Wang J, Zhu M, Chen Y, *et al*. Muller cell-derived VEGF is a significant contributor to retinal neovascularization. J Pathol 2009;219:446-54.
26. Wang J, Xu X, Elliott MH, Zhu M, Le YZ. Muller cell-derived VEGF is essential for diabetes-induced retinal inflammation and vascular leakage. Diabetes 2010;59:2297-305.
27. Wirostko B, Wong TY, Simo R. Vascular endothelial

growth factor and diabetic complications. Prog Retin Eye Res 2008;27:608-21.
28. Harhaj NS, Felinski EA, Wolpert EB, Sundstrom JM, Gardner TW, Antonetti DA. VEGF activation of protein kinase C stimulates occludin phosphorylation and contributes to endothelial permeability. Invest Ophthalmol Vis Sci 2006;47:5106-15.
29. Murakami T, Felinski EA, Antonetti DA. Occludin phosphorylation and ubiquitination regulate tight junction trafficking and vascular endothelial growth factor-induced permeability. J Biol Chem 2009;284:21036-46.
30. PKC-DRS2 Group, Aiello LP, Davis MD, Girach A, Kles KA, Milton RC, *et al*. Effect of ruboxistaurin on visual loss in patients with diabetic retinopathy. Ophthalmology 2006;113:2221-30.
31. Navaratna D, McGuire PG, Menicucci G, Das A. Proteolytic degradation of VE-cadherin alters the blood-retinal barrier in diabetes. Diabetes 2007;56:2380-7.
32. Enge M, Bjarnegård M, Gerhardt H, Gustafsson E, Kalén M, Asker N, *et al*. Endothelium-specific platelet-derived growth factor-B ablation mimics diabetic retinopathy. EMBO J 2002;21:4307-16.
33. Geraldes P, Hiraoka-Yamamoto J, Matsumoto M, Clermont A, Leitges M, Marette A, *et al*. Activation of PKC-delta and SHP-1 by hyperglycemia causes vascular cell apoptosis and diabetic retinopathy. Nat Med 2009;15:1298-306.
34. Hotamisligil GS. Inflammation and metabolic disorders. Nature 2006;444:860-7.
35. Wilkinson-Berka JL, Tan G, Jaworski K, Miller AG. Identification of a retinal aldosterone system and the protective effects of mineralocorticoid receptor antagonism on retinal vascular pathology. Circ Res 2009;104:124-33.
36. Demircan N, Safran BG, Soylu M, Ozcan AA, Sizmaz S. Determination of vitreous interleukin-1 (IL-1) and tumour necrosis factor (TNF) levels in proliferative diabetic retinopathy. Eye (Lond) 2006;20:1366-9.
37. Felinski EA, Cox AE, Phillips BE, Antonetti DA. Glucocorticoids induce transactivation of tight junction genes occludin and claudin-5 in retinal endothelial cells via a novel cis-element. Exp Eye Res 2008;86:867-78.
38. Guo M, Ricardo SD, Deane JA, Shi M, Cullen-McEwen L, Bertram JF. A stereological study of the renal glomerular vasculature in the db/db mouse model of diabetic nephropathy. J Anat 2005;207:813-21.
39. Nakagawa T, Kosugi T, Haneda M, Rivard CJ, Long DA. Abnormal angiogenesis in diabetic nephropathy. Diabetes 2009;58:1471-8.
40. Hohenstein B, Hausknecht B, Boehmer K, Riess R, Brekken RA, Hugo CP. Local VEGF activity but not VEGF expression is tightly regulated during diabetic nephropathy in man. Kidney Int 2006;69:1654-61.
41. McGinn S, Saad S, Poronnik P, Pollock CA. High glucose-mediated effects on endothelial cell proliferation occur via p38 MAP kinase. Am J Physiol Endocrinol Metab 2003;285:E708-17.
42. Osterby R, Bangstad HJ, Nyberg G, Rudberg S. On glomerular structural alterations in type-1 diabetes. Companions of early diabetic glomerulopathy. Virchows Arch 2001;438:129-35.
43. Khazaei M, Fallahzadeh AR, Sharifi MR, Afsharmoghaddam N, Javanmard SH, Salehi E. Effects of diabetes on myocardial capillary density and serum angiogenesis biomarkers in male rats. Clinics (Sao Paulo) 2011;66:1419-24.
44. Fallahzadeh AR, Khazaei M, Sharifi MR. Restoration of angiogenesis by enalapril in diabetic hindlimb ischemic rats. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2011;155:137-42.
45. Nakagawa T, Sato W, Glushakova O, Heinig M, Clarke T, Campbell-Thompson M, *et al*. Diabetic endothelial nitric oxide synthase knockout mice develop advanced diabetic nephropathy. J Am Soc Nephrol 2007;18:539-50.
46. Woolf AS, Gnudi L, Long DA. Roles of angiopoietins in kidney development and disease. J Am Soc Nephrol 2009;20:239-44.
47. Yuan HT, Khankin EV, Karumanchi SA, Parikh SM. Angiopoietin 2 is a partial agonist/antagonist of Tie2 signaling in the endothelium. Mol Cell Biol 2009;29:2011-22.
48. De Vriese AS, Tilton RG, Elger M, Stephan CC, Kriz W, Lameire NH. Antibodies against vascular endothelial growth factor improve early renal dysfunction in experimental diabetes. J Am Soc Nephrol 2001;12:993-1000.
49. Carmeliet P, Storkebaum E. Vascular and neuronal effects of VEGF in the nervous system: Implications for neurological disorders. Semin Cell Dev Biol 2002;13:39-53.
50. Kim H, Park JS, Choi YJ, Kim MO, Huh YH, Kim SW, *et al*. Bone marrow mononuclear cells have neurovascular tropism and improve diabetic neuropathy. Stem Cells 2009;27:1686-96.
51. Malik RA, Tesfaye S, Newrick PG, Walker D, Rajbhandari SM, Siddique I, *et al*. Sural nerve pathology in diabetic patients with minimal but progressive neuropathy. Diabetologia 2005;48:578-85.
52. Yagihashi S, Yamagishi SI, Wada Ri R, Baba M, Hohman TC, Yabe-Nishimura C, *et al*. Neuropathy in diabetic mice overexpressing human aldose reductase and effects of aldose reductase inhibitor. Brain 2001;124:2448-58.
53. Altan VM. The pharmacology of diabetic complications. Curr Med Chem 2003;10:1317-27.
54. Lazarovici P, Marcinkiewicz C, Lelkes PI. Cross talk

between the cardiovascular and nervous systems: Neurotrophic effects of vascular endothelial growth factor (VEGF) and angiogenic effects of nerve growth factor (NGF)-implications in drug development. Curr Pharm Des 2006;12:2609-22.
55. Ii M, Nishimura H, Kusano KF, Qin G, Yoon YS, Wecker A, *et al*. Neuronal nitric oxide synthase mediates statin-induced restoration of vasa nervorum and reversal of diabetic neuropathy. Circulation 2005;112:93-102.
56. Zacchigna S, Lambrechts D, Carmeliet P. Neurovascular signalling defects in neurodegeneration. Nat Rev Neurosci 2008;9:169-81.
57. Vincent AM, Russell JW, Low P, Feldman EL. Oxidative stress in the pathogenesis of diabetic neuropathy. Endocr Rev 2004;25:612-28.
58. Galeano M, Torre V, Deodato B, Campo GM, Colonna M, Sturiale A, *et al*. Raxofelast, a hydrophilic vitamin E-like antioxidant, stimulates wound healing in genetically diabetic mice. Surgery 2001;129:467-77.
59. Trautner C, Haastert B, Spraul M, Giani G, Berger M. Unchanged incidence of lower-limb amputations in a German City, 1990-1998. Diabetes Care 2001;24:855-9.
60. Trautner C, Haastert B, Giani G, Berger M. Amputations and diabetes: A case-control study. Diabet Med 2002;19:35-40.
61. Lerman OZ, Galiano RD, Armour M, Levine JP, Gurtner GC. Cellular dysfunction in the diabetic fibroblast: Impairment in migration, vascular endothelial growth factor production, and response to hypoxia. Am J Pathol 2003;162:303-12.
62. Maruyama K, Asai J, Ii M, Thorne T, Losordo DW, D'Amore PA. Decreased macrophage number and activation lead to reduced lymphatic vessel formation and contribute to impaired diabetic wound healing. Am J Pathol 2007;170:1178-91.
63. Verheul HM, Pinedo HM. Possible molecular mechanisms involved in the toxicity of angiogenesis inhibition. Nat Rev Cancer 2007;7:475-85.
64. Kolluru GK, Bir SC, Kevil CG. Endothelial dysfunction and diabetes: Effects on angiogenesis, vascular remodeling, and wound healing. Int J Vasc Med 2012;2012:918267.
65. Kusumanto YH, van Weel V, Mulder NH, Smit AJ, van den Dungen JJ, Hooymans JM, *et al*. Treatment with intramuscular vascular endothelial growth factor gene compared with placebo for patients with diabetes mellitus and critical limb ischemia: A double-blind randomized trial. Hum Gene Ther 2006;17:683-91.
66. Mohammad G, pandey HP, Tripathi K. Diabetic wound healing and its angiogenesis with special reference to nanoparticles. Dig J Nanomater Biostructures 2008;3:203-8.
67. Mishra VK, Mohammad G, Mishra SK. Down regulation of telomerase activity may enhance by Nanoparticle mediated curcumin delivery. Dig J Nanomater Biostructures 2008;3:163-9.
68. Polednak AP. Estimating the number of U.S. incident cancers attributable to obesity and the impact on temporal trends in incidence rates for obesity-related cancers. Cancer Detect Prev 2008;32:190-9.
69. Vigneri P, Frasca F, Sciacca L, Pandini G, Vigneri R. Diabetes and cancer. Endocr Relat Cancer 2009;16:1103-23.
70. Barone BB, Yeh HC, Snyder CF, Peairs KS, Stein KB, Derr RL, *et al*. Long-term all-cause mortality in cancer patients with preexisting diabetes mellitus: A systematic review and meta-analysis. JAMA 2008;300:2754-64.
71. Mardilovich K, Pankratz SL, Shaw LM. Expression and function of the insulin receptor substrate proteins in cancer. Cell Commun Signal 2009;7:14.
72. Johnson JA, Pollak M. Insulin, glucose and the increased risk of cancer in patients with type 2 diabetes. Diabetologia 2010;53:2086-8.
73. Pollak M. Insulin and insulin-like growth factor signalling in neoplasia. Nat Rev Cancer 2008;8:915-28.
74. Yun J, Rago C, Cheong I, Pagliarini R, Angenendt P, Rajagopalan H, *et al*. Glucose deprivation contributes to the development of KRAS pathway mutations in tumor cells. Science 2009;325:1555-9.
75. Heikkilä K, Harris R, Lowe G, Rumley A, Yarnell J, Gallacher J, *et al*. Associations of circulating C-reactive protein and interleukin-6 with cancer risk: Findings from two prospective cohorts and a meta-analysis. Cancer Causes Control 2009;20:15-26.
76. Balkwill F. TNF-alpha in promotion and progression of cancer. Cancer Metastasis Rev 2006;25:409-16.
77. Katiyar SK, Meeran SM. Obesity increases the risk of UV radiation-induced oxidative stress and activation of MAPK and NF-kappaB signaling. Free Radic Biol Med 2007;42:299-310.
78. van Kruijsdijk RC, van der Wall E, Visseren FL. Obesity and cancer: The role of dysfunctional adipose tissue. Cancer Epidemiol Biomarkers Prev 2009;18:2569-78.
79. Andreasen PA, Egelund R, Petersen HH. The plasminogen activation system in tumor growth, invasion, and metastasis. Cell Mol Life Sci 2000;57:25-40.
80. Sakakibara T, Hibi K, Koike M, Fujiwara M, Kodera Y, Ito K, *et al*. Plasminogen activator inhibitor-1 as a potential marker for the malignancy of colorectal cancer. Br J Cancer 2005;93:799-803.
81. Hilska M, Roberts PJ, Collan YU, Laine VJ, Kössi J, Hirsimäki P, *et al*. Prognostic significance of matrix metalloproteinases-1, -2, -7 and -13 and tissue inhibitors of metalloproteinases-1, -2, -3 and -4 in colorectal cancer. Int J Cancer 2007;121:714-23.
82. Cho YB, Lee WY, Song SY, Shin HJ, Yun SH, Chun HK. Matrix metalloproteinase-9 activity is associated with

poor prognosis in T3-T4 node-negative colorectal cancer. Hum Pathol 2007;38:1603-10.
83. Tian M, Cui YZ, Song GH, Zong MJ, Zhou XY, Chen Y, *et al*. Proteomic analysis identifies MMP-9, DJ-1 and A1BG as overexpressed proteins in pancreatic juice from pancreatic ductal adenocarcinoma patients. BMC Cancer 2008;8:241.
84. Roy R, Yang J, Moses MA. Matrix metalloproteinases as novel biomarkers and potential therapeutic targets in human cancer. J Clin Oncol 2009;27:5287-97.
85. Stattin P, Lukanova A, Biessy C, Söderberg S, Palmqvist R, Kaaks R, *et al*. Obesity and colon cancer: Does leptin provide a link? Int J Cancer 2004;109:149-52.
86. Tamakoshi K, Toyoshima H, Wakai K, Kojima M, Suzuki K, Watanabe Y, *et al*. Leptin is associated with an increased female colorectal cancer risk: A nested case-control study in Japan. Oncology 2005;68:454-61.
87. Munzberg H, Myers MG, Jr. Molecular and anatomical determinants of central leptin resistance. Nat Neurosci 2005;8:566-70.
88. Hoda MR, Keely SJ, Bertelsen LS, Junger WG, Dharmasena D, Barrett KE. Leptin acts as a mitogenic and antiapoptotic factor for colonic cancer cells. Br J Surg 2007;94:346-54.
89. Guiu B, Petit JM, Bonnetain F, Ladoire S, Guiu S, Cercueil JP, *et al*. Visceral fat area is an independent predictive biomarker of outcome after first-line bevacizumab-based treatment in metastatic colorectal cancer. Gut 2010;59:341-7.
90. Silha JV, Krsek M, Sucharda P, Murphy LJ. Angiogenic factors are elevated in overweight and obese individuals. Int J Obes (Lond) 2005;29:1308-14.
91. Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: Epidemiology, pathophysiology, and management. JAMA 2002;287:2570-81.
92. Haffner SM, Lehto S, Ronnemaa T, Pyorala K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. N Engl J Med 1998;339:229-34.
93. De Vriese AS, Verbeuren TJ, Van de Voorde J, Lameire NH, Vanhoutte PM. Endothelial dysfunction in diabetes. Br J Pharmacol 2000;130:963-74.
94. Khazaei M, Mobarakeh JI, Rahimi AA, Razavi MR. Effect of chronic L-Arginine supplementation on aortic fatty streak formation and serum nitric oxide concentration in normal and high-cholesterol fed rabbits. Acta Physiol Hung 2012;99:87-93.
95. Ceriello A, Quagliaro L, D'Amico M, Di Filippo C, Marfella R, Nappo F, *et al*. Acute hyperglycemia induces nitrotyrosine formation and apoptosis in perfused heart from rat. Diabetes 2002;51:1076-82.
96. Yin JH, Yang DI, Ku G, Hsu CY. iNOS expression inhibits hypoxia-inducible factor-1 activity. Biochem Biophys Res Commun 2000;279:30-4.
97. Cooke JP, Losordo DW. Nitric oxide and angiogenesis. Circulation 2002;105:2133-5.
98. Marfella R, Esposito K, Nappo F, Siniscalchi M, Sasso FC, Portoghese M, *et al*. Expression of angiogenic factors during acute coronary syndromes in human type 2 diabetes. Diabetes 2004;53:2383-91.
99. Uemura S, Matsushita H, Li W, Glassford AJ, Asagami T, Lee KH, *et al*. Diabetes mellitus enhances vascular matrix metalloproteinase activity: Role of oxidative stress. Circ Res 2001;88:1291-8.
100. Inoguchi T, Li P, Umeda F, Yu HY, Kakimoto M, Imamura M, *et al*. High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C-dependent activation of NAD (P) H oxidase in cultured vascular cells. Diabetes 2000;49:1939-45.
101. Sasso FC, Torella D, Carbonara O, Ellison GM, Torella M, Scardone M, *et al*. Increased vascular endothelial growth factor expression but impaired vascular endothelial growth factor receptor signaling in the myocardium of type 2 diabetic patients with chronic coronary heart disease. J Am Coll Cardiol 2005;46:827-34.
102. Chou E, Suzuma I, Way KJ, Opland D, Clermont AC, Naruse K, *et al*. Decreased cardiac expression of vascular endothelial growth factor and its receptors in insulin-resistant and diabetic States: A possible explanation for impaired collateral formation in cardiac tissue. Circulation 2002;105:373-9.
103. Sasso FC, Carbonara O, Persico E, D'Ambrosio R, Coppola L, Nasti R, *et al*. Increased vascular endothelial growth factor mRNA expression in the heart of streptozotocin-induced diabetic rats. Metabolism 2003;52:675-8.
104. Zhang X, Lassila M, Cooper ME, Cao Z. Retinal expression of vascular endothelial growth factor is mediated by angiotensin type 1 and type 2 receptors. Hypertension 2004;43:276-81.
105. Shiojima I, Walsh K. Role of Akt signaling in vascular homeostasis and angiogenesis. Circ Res 2002;90:1243-50.
106. Kureishi Y, Luo Z, Shiojima I, Bialik A, Fulton D, Lefer DJ, *et al*. The HMG-CoA reductase inhibitor simvastatin activates the protein kinase Akt and promotes angiogenesis in normocholesterolemic animals. Nat Med 2000;6:1004-10.

Source of Support: Nil, **Conflict of Interest:** None declared.