Metabolic syndrome impairs notch signaling and promotes apoptosis in chronically ischemic myocardium

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Objective: Impaired angiogenesis is a known consequence of metabolic syndrome (MetS); however, the mechanism is not fully understood. Recent studies have shown that the notch signaling pathway is an integral component of cardiac angiogenesis. We tested, in a clinically relevant swine model, the effects of MetS on notch and apoptosis signaling in chronically ischemic myocardium.

Methods: Ossabaw swine were fed either a regular diet (control [CTL], n = 8) or a high-cholesterol diet (MetS, n = 8) to induce MetS. An ameroid constrictor was placed to induce chronic myocardial ischemia. Eleven weeks later, the wine underwent cardiac harvest of the ischemic myocardium.

Results: Downregulation of pro-angiogenesis proteins notch2, notch4, jagged2, angiopoietin 1, and endothelial nitric oxide synthase were found in the MetS group compared with the CTL group. Also, upregulation of pro-apoptosis protein caspase 8 and downregulation of anti-angiogenesis protein phosphorylated forkhead box transcription factor 03 and pro-survival proteins phosphorylated P38 and heat shock protein 90 were present in the MetS group. Cell death was increased in the MetS group compared with the CTL group. Both CTL and MetS groups had a similar arteriolar count and capillary density, and notch3 and jagged1 were both similarly concentrated in the smooth muscle wall.

Conclusions: MetS in chronic myocardial ischemia significantly impairs notch signaling by downregulating notch receptors, ligands, and pro-angiogenesis proteins. MetS also increases apoptosis signaling, decreases survival signaling, and increases cell death in chronically ischemic myocardium. Although short-term angiogenesis appears unaffected in this model of early MetS, the molecular signals for angiogenesis are impaired, suggesting that inhibition of notch signaling might underlie the decreased angiogenesis in later stages of MetS. (J Thorac Cardiovasc Surg 2014;148:1048-55)

The metabolic syndrome (MetS) is a cluster of metabolic derangements that includes obesity, insulin resistance, glucose intolerance, dyslipidemia, and hypertension. MetS substantially increases the risk of cardiovascular disease and mortality.[1,2] Despite advancements in surgical technique and percutaneous interventions, patients with hypertension, hyperlipidemia, and diabetes have had significantly greater mortality after angioplasty, coronary stenting, and coronary artery bypass grafting.[3] The promising results from animal studies in therapeutic revascularization with growth factor, gene, or cell therapy have met with disappointing results in humans, with minimal clinical improvements in myocardial angiogenesis.[4] Not surprisingly, young healthy animals with chronic myocardial ischemia are better able to adapt to the ischemic insult, but older patients with multiple comorbidities, including MetS, have limited myocardial adaptability to ischemia. The discordant results between positive findings from animal studies and negative clinical results highlights the need to understand the effects of MetS on the molecular angiogenic signaling pathways using a clinically relevant animal model.

Ossabaw miniswine are a breed of feral pigs that were isolated on Ossabaw Island off the coast of Georgia almost 500 years ago by Spanish explorers. This pig breed has been identified as an excellent model for MetS owing to its "thrifty genotype," which allowed these pigs to adapt to the harsh island conditions by storing large amounts of fat during the feasting period.[5] When sedentary and fed a high-fat, high-calorie, atherogenic diet, these swine develop profound obesity and all the hallmark features of MetS and are, thus, a useful model to study MetS and coronary artery disease.[5-7]

We recently demonstrated that animals with chronic myocardial ischemia and perivascular vascular endothelial growth factor (VEGF) had significantly improved

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Abbreviations and Acronyms

Ang1 = angiopoietin 1
DLL = delta like ligand
eNOS = endothelial nitric oxide synthase
MetS = metabolic syndrome
NIH = National Institutes of Health
SMA = smooth muscle actin
TUNEL = terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end labeling
VEGF = vascular endothelial growth factor
VEGFR = VEGF receptor

neovascularization and Notch receptor and ligand expression.[8] The Notch signaling pathway is an evolutionarily conserved pathway important for many processes, including cell fate determination differentiation, proliferation, apoptosis, and regeneration.[9] Studies have shown that postnatal Notch signaling is critical for angiogenesis.[10,11] Interest is growing in the clinical utility of Notch modulators to suppress angiogenesis in tumors and promote angiogenesis in ischemic myocardium.[12] Although research in the role of Notch signaling in developmental biology is longstanding, the role of Notch signaling in mature myocardium in response to ischemia and MetS is largely unknown. The purpose of the present study was to examine the effects of MetS on Notch signaling in response to chronic myocardial ischemia in the clinically relevant Ossabaw model of early MetS.

METHODS

Animal Model

Sixteen intact male Ossabaw miniswine (Purdue Ossabaw Facility, Indiana University, Indianapolis, Ind) were split into 2 groups according to diet at 6 weeks of age. The control (CTL) group was fed 500 g/d of regular chow (n = 8). The high-cholesterol swine were fed 500 g/d of high-cholesterol chow (MetS group, n = 8) consisting (by weight) of 4% cholesterol, 17.2% coconut oil, 2.3% corn oil, 1.5% sodium cholate, and 75% regular chow (Sinclair Research, Columbia, Mo). After 9 weeks of diet initiation, all the swine underwent surgical placement of an ameroid constrictor to induce chronic myocardial ischemia (see "Surgical Interventions"). Postoperatively, all the swine continued their respective diets. Eleven weeks after ameroid constrictor placement, all the swine underwent euthanasia and cardiac tissue harvest. All the swine were observed to ensure complete consumption of food and unlimited access to water and were housed in a warm nonstressful environment for the duration of the experiment.

Surgical Interventions

Anesthesia. Anesthesia was induced with an intramuscular injection of telazol (4.4 mg/kg). The swine were endotracheally intubated and mechanically ventilated at 12 to 20 breaths/min. General anesthesia was maintained with a gas mixture of oxygen at 1.5 to 2 L/min and 0.75% to 3.0% isoflurane.

Ameroid constrictor placement. For ameroid constrictor placement, the swine were given a single dose of intravenous enrofloxacin (5 mg/kg) for antibiotic prophylaxis, and general anesthesia was induced and maintained. The swine were prepared and draped in the usual sterile fashion. The heart was exposed through a left minithoracotomy through the fourth intercostal space and pericardiotomy. The left atrial appendage was retracted, and the left circumflex artery was dissected at the take off of the left main coronary artery. A titanium ameroid constrictor (1.75-2.25 mm internal diameter) ameroid constrictor was placed around the proximal left circumflex artery, just after its take off from the left main coronary artery (Research Instruments SW, Escondito, Calif). The pericardium was loosely reapproximated with interrupted 4-0 Nurolon sutures (Ethicon, Somerville, NJ), followed by a layered closure of the surgical incision. Postoperative pain was controlled by a single dose of intramuscular buprenorphine (0.03 mg/kg) and a 72-hour fentanyl patch (4 μg/kg). All the swine received 325 mg of aspirin daily starting 1 day preoperatively and continuing for 5 days for prophylaxis against thromboembolic events. All the swine continued perioperative antibiotics (enrofloxacin 68 mg orally daily for 5 days).

Cardiac harvest. For cardiac harvest, with the swine under general anesthesia, the heart was exposed by way of a median sternotomy, and the swine were euthanized by exsanguination. Cardiac tissue from the ischemic territory in the left circumflex artery distribution was collected for additional analysis. The institutional animal care and use committee of the Rhode Island Hospital approved all experiments. The swine were cared for in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health [NIH] publication no. 5377-3, 1996).

Immunohistochemical Staining for Angiogenesis

For immunohistochemical staining for angiogenesis, frozen myocardium was sectioned (10-μm thickness) and fixed in 10% formalin for 10 minutes. The sections were blocked with 1% bovine serum albumin in phosphate-buffered saline for 1 hour at room temperature and incubated with antibodies against the porcine endothelial marker CD31 (R&D Systems, Minneapolis, Minn) and smooth muscle actin (SMA) (Sigma-Aldrich, St Louis, Mo), followed by the appropriate Alexa-Fluor conjugated antibody (Jackson ImmunoResearch, West Grove, Pa) for 45 minutes. Slides were then mounted with 46′-diamidino-2-phenylindole-2 HCl–containing medium (Vectashield; Vector Laboratories, Burlingame, Calif). Images were captured at ×20 magnification with a Nikon E800 Eclipse microscope (Nikon, Tokyo, Japan) at the same exposure in 3 random fields. The capillaries were defined as structures 5 to 25 μm^2 in cross-sectional area, and arterioles were defined by co-localization of SMA (red) and CD31 (green) staining. The arteriolar and capillary density were measured using Image J software (NIH, Bethesda, Md). The percentage of capillary and arteriolar density for each pig was averaged from the 3 randomly selected myocardial tissue sections.

Protein Expression

To determine protein expression, 40 μg of the radioimmunoprecipitation assay (Boston BioProducts, Ashland, Mass) soluble fraction of myocardial lysates was fractionated using sodium dodecyl sulfate polyacrylamide gel electrophoresis 3% to 8% Tris-acetate gel (NuPage Novex Mini Gel; Invitrogen, Carlsbad, Calif) for molecular weight targets >100 kDa and 4% to 12% Bis-Tris gels for molecular weight targets <100 kDa (NuPage Novex Mini Gel; Invitrogen). The protein was then transferred to polyvinylidene difluoride membranes (Millipore, Billerica, Mass) and incubated overnight at 4°C with primary antibodies at the dilutions recommended by the manufacturer against Notch1, Notch2, Notch3, Notch4, Jagged1, Jagged2, VEGF receptor (VEGFR)2, vascular endothelial cadherin, endothelial nitric oxide synthase (eNOS),

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