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MMPs inhibition as molecular approach to aneurysm treatment

Aortic aneurysm is a degenerative disease characterized by destruction of aortic architecture and subsequent dilatation. Because of its silent nature, the likely consequence of undiagnosed aneurysms includes rupture, which can be fatal and requires emergency surgical management. Aortic aneurysm and dissections account for 1% to 2% of all deaths in the Western countries and the incidence of thoracic aortic aneurysm (TAA) is ≈10.4 per 100 000 person years. (Lindsay et al, 2011; Ramanath et al, 2009). Aortic aneurysms are categorized into two main groups depending on their location: thoracic aortic aneurysms (TAAs) and abdominal aortic aneurysms (AAAs). AAAs are the most common group and are connected with various lifestyle-associated risk factors, including hyperlipidemia, hypertension, sex and age. However, smoking is generally found as one of the most important risk factor (Blanchard et al, 2000; Lederle et al, 1997). Heritability estimates as high as 70% have been found, and recently, several genes and loci have been associated with abdominal aortic aneurysms (DAB2IP, LRP1, CDKN2B-AS1, CNTN3, LPA, IL6R, and the sortilin-1 SORT1 locus). (Wahlgren et al, 2010; Gretarsdottir et al, 2010;Bown et al, 2011; Helgadottir et al, 2008; Bown et al, 2008; Elmore et al, 2009; Hinterseher et al, 2011; Harrison et al, 2013; Jones et al, 2013; Helgadottir et al, 2012). Aneurysm is characterized by medial degeneration. Medial disruption is determined by disaggregation of the lamellar organization of elastic fibers, accumulation of basophilic ground substance with cystic lesion, degradation of the extracellular matrix (ECM), including both elastin fragmentation and disruptions of collagen, and apoptosis of vascular smooth muscle cells (VSMC) (Yuan et al, 2011). One of the reasons of aneurysm development is the involvement of matrix metalloprotinases (MMPs). MMPs belong to the family of proteolytic enzymes that degrade several components of ECM. Under normal physiological conditions, the activities of MMPs are regulated at the level of transcription, at the level of activation of the precursor zymogens, and at the level of interaction with specific ECM components. Also, endogenous tissue inhibitors of MMPs (TIMPs) provide a balancing mechanism to prevent excessive degradation of ECM. An imbalance between MMPs and TIMPs could lead to increase the MMPs activity and lead to pathological changes in the vessel wall structure associated with vascular disease (Raffetto and Khalil, 2008). The mammalian MMPs are a group of 23 structurally related enzymes that have a catalytic Zn^{2+} ion (Nagase et al, 2006; Visse and Nagase, 2003). All MMPs are synthetized as prepro-enzymes and secreted as inactive pro-MMPs. The pro-peptide domain (about 80 amino acids) has a conserved unique PRCG(V/N)PD sequence. The Cys within this sequence (the "cysteine swith") ligates the catalytic zinc to maintain the latency of pro-MMPs (VanWart et al, 1990; Becker et al, 1995). The catalytic domain (about 170aa) contains a zinc binding motif HEXXHXXGXXH and a conserved methionine, which forms a unique "Met-turn" structure. (Bode et al, 1993). This domain consists of a five-stranded β-sheet, three α-helices, and bridging loops. (Dhanaraj et al, 1996). MMP2 and MMP9 have three repeats of fibronectin-type II domain inserted in the catalytic domain. These repeats interact with collagens and gelatins (Allan et al, 1995; Steffensen et al, 1995). The C-terminal hemopexin-like domain (about 210 aa) has an ellipsoidal disk shape with a four bladed β-propeller structure; each blade consists of four antiparallel β-strands and an α-helix (Gomis-Ruth et al, 1996). The hemopexin domain is required for collagenases to cleave triple helical interstitial collagens, although the catalytic domain alone retains proteolytic activity toward to other substrates. The hemopexin domain of MMP2 is also required for the cell-surface activation of pro-MMP-2 by MT1-MMP (Murphy et al, 1992; Strongin et al, 1995). MMPs have overlapping specificities for structural ECM components but could be grouped into interstitial collagenases (MMPs-1, -8, -13, -14) that cleave fibrillar collagens, gelatinases (MMPs-2 and -9) that efficiently cleave denatured collagen (i.e. gelatin) and stromelysins (MMPs-3, -7, -10, -11) that have a broad specificity but do not cleave intact fibrillar collagen. Matrix metalloelastase (MMP-12), which is faintly genetically related to other MMPs, cleaves other ECM components as well as elastin (Nagase et al, 2006; Visse and Nagase, 2003). In principle, a combination of MMPs therefore has the ability to extensively degrade the ECM. In addition, many of the MMPs have the ability to cleave and activate the pro-forms of other MMPs, thereby acting in protease cascades that could amplify their effectiveness (Nagase et al, 2006; Visse and Nagase, 2003). Several MMPs remain attached to the membrane (e.g. MMP-14 to -17, -25, and -26). For these reason they are called membrane-type MMPs (MT-MMPs). Some soluble MMPs, together with MT-MMPs, have indirect cell surface attachments which arise them in a privileged position to degrade pericellular ECM components and act as shredder (Nagase et al, 2006; Visse and Nagase, 2003). Indeed, many MMPs have the ability to cleave cell surface proteins, modify the activity of secreted proteins and release factors sequestered in the pericellular ECM (Nagase et al, 2006; Visse and Nagase, 2003). One of the features of MMP is that many of those genes are "inducibile" by a group of effectors such as growth factors, cytokines, chemical agents, physical stress, and oncogenic cellular transformation. Furthermore, pathological observations suggest that the earliest events may be the loss of elastin mediated by VSMCs, perhaps as a result of inflammatory activation, then followed by influx of leukocytes in response to the production of ECM fragments with chemotactic activities. This suggests a multi-stage, initiation and destabilization, paradigm for aneurysm growth and rupture. Initial loss of elastin in the medial layer causes compensatory fibrosis leading to normal or increased collagen deposition and consequently the loss the mechanical properties of the wall. Later, destruction of all major matrix components, as a consequence of inflammation, causes further distension and eventually rupture of the vessel wall. Consistent with this,
imaging studies and pathological examinations of advanced AAAs demonstrate an association between leukocyte infiltration and likelihood of rupture (Hong et al, 2010; Reeps et al, 2008). MMP-2s are produced by SMCs, among other cells, in the medial wall. However this matrixins is a constitutive enzyme produced by multiple cell types, activated by membrane-bound membrane type-1 (MT1-MMP), and inhibited by tissue inhibitor of metalloproteinases type 2 (TIMP-2). MMP-2 has substrate specificity for elastin and fibrillar collagen (Aimes et al, 1995) and is found in the normal and aneurysm aorta in association with MT1-MMP and TIMP-2 (Crowther et al, 2000). Longo and colleagues suggested a critical role for smooth muscle cells (SMCs) in experimental aneurysm formation (Longo et al, 2002). MMP-2 knockout mice are resistant to experimental aortic aneurysms using a model of abluminal calcium chloride application. Reconstitution of these mice with wild-type bone marrow did not alter this resistance, suggesting that

MMP-2 derived from outside the bone marrow is critical (Jones et al, 2009). In aneurysm tissue there is an increase of MMP-2 production resulted from increased MMP-2 transcription, with comparable levels of TIMP-2 and MT1-MMP mRNA. These data suggested the regulation of MMP-2 gene expression was altered in aortic SMCs from patients with abdominal aortic aneurysm (Goodall et al, 2001). Moreover, different studies have revealed that also inflammatory processes play a key role in the development of AAAs, which involves the infiltration of various immune cells (particularly macrophages and T cells) (Longo et al 2002; Xiong et al 2004; Eliason et al 2005). MMP9 derived from macrophages and MMP2 derived from vascular smooth muscle cells (SMCs) (Weintraub et al 2009; Sakalihasan et al 2005; Longo et al 2002) have been shown to be critical factors required for the elastin destruction and proteolytic degradation, that are hallmark features of AAAs, thereby leading to gradual aortic dilatation. Interestingly, such vascular wall degradation in human AAAs is often also accompanied by calcification of the aneurysmal wall, suggesting a possible link between aneurysm formation and calcification (Siegel et al 1994). The object of developing inhibitors of MMPs and inflammation is to stabilize in an early stage aneurysmal disease, and prevent further expansion, in order to avoid the surgery. From this viewpoint, a pharmacological strategy to inhibit MMPs activity might prevent the progression from asymptomatic aneurysm to critical large aneurysm. Several studies have postulated a pivotal role of MMP-2 in early aneurysm formation, while it was demonstrated that MMP-9 was involved in aneurysm expansion at a large stage (Freeston at al, 1995; Thomson et al, 1995). One of the main methods to inhibits MMPs, is the use of RNA interference technique, where a small molecule of RNA (about 20-25 bp) inhibits gene expression by causing the destruction of mRNA molecule encoding the target protein. RNA interference (siRNA) specifically suppresses gene expression in mammalian cells in vitro. This inhibitory effects results from selective degradation of target mRNA (McManus et al 2002). Recent studies show that siRNA can be used for inhibition of gene expression *in vivo*. These results set the path for therapeutic application, either locally or systematically (Dorn et al, 2004; Soutschek et al, 2004). MMP-2 and MMP-9 are interesting target for siRNA- or shRNA-based on artificial therapy, since MMP-2 is constitutively expressed in SMCs, and MMP-9 is expressed by inflammatory cells. An ideal siRNA delivery vehicle for systemic administration should have long circulation time in blood, to facilitate accumulation at target site, and be sufficiently internalized into target cells for high-efficiency of gene silencing. One of our goals is to inhibit MMP2 and MMP9 expression through the use of siRNA, carried in the desired site exploiting a nanotechnological approach. We would like to use nanoparticles that can be activated by our target proteins, such as MMP2 and MMP9. Indeed, our target tissue is rich of these metalloproteinases, and these nanoparticles can be uptaken only after proteolytic degradation of the polymeric molecules, that are inhibitor of uptaking. (Wang et al 2014).

Methods

Mouse model of abdominal aortic aneurysm (AAA)

AAA was induced in C57BL/6 mice by periaortic application of $CaCl_2$ (AAA group). PBS-treated mice were used as a sham control (SHAM group). The infrarenal aorta above the iliac bifurcation of mice was treated with calcium chloride or PBS. Anesthetized mice Xilazine (15 mg/kg), Ketamin (100 mg/kg) e Buprenorphine (0.05 mg/kg) using intraperitoneal injection were placed under an operating microscope Leica. A long midline abdominal incision from pubis to xiphoid was made, and the abdominal cavity was exposed. The intestines were retracted superiorly and covered with wet gauze to keep them moist during the operation. The abdominal aorta was exposed and a gauze was placed on the aorta, soaked with a solution of $CaCl_2$ (0.25M or 0.5M) or PBS, used as control, for 15 minutes (Wang et al, 2013). The intestines were then returned to the abdominal cavity. The abdominal incision was closed with continuous running 5-0 nylon suture, and the mice were placed in warming cages to recover. At 4 weeks after the operation, aortic tissue was excised for further examination.

Histological staining and Immunohistochemistry

Samples were collected from different donors. Tissue samples were washed in phosphate-buffered saline (PBS) and fixed in 4% formalin for 48h at 4° C. The samples were subjected to several dehydration steps of 60 min incubation in solutions of increasing ethanol content (50%, 70%, 95% and 100%) and 1h incubation in xylene. The specimens were embedded in paraffin (Sherwood Medical, St. Louis, MO, USA) and serial sections were cut with a microtome (Leica- Jung, Germany) in consecutive 5 mm-thick sections. Rehydrated sections were stained with haematoxylin and eosin and Weigert (for elastin detection). Immunohistochemistry was performed on deparaffinized sections. Samples were processed with primary MMP2 and MMP9 antibodies, followed by a secondary antibody (Vector, CA, USA) incubation. The reaction was detected using the avidin–biotin method.

Immunofluorescence

Immunohistochemistry was performed on deparaffinized sections. Samples were processed with primary anti-elastin antibody (Diagnostic Biosystems, CA, USA) followed by a secondary antibody-FITC (Vector, CA, USA) incubation.

Gelatin zymography for detection of MMP2 and MMP9

Samples were collected from different donors. Non-reducted conditioned media samples were resolved by SDS-PAGE gels containing gelatin. After electrophoresis, gels were incubated with TRITON X-100 for 3h at room temperature, and then incubated in a solution of $CaCl_2$ (1mM) and NaCl (15mM), pH 7.4 overnight at 37°C. After incubation, gels were fixed and then gels were visualized with Coomassie stain.

Western blot

Cells were lysed in hot SDS buffer (1% w/v SDS, 100 mM Tris–HCl, pH 7.0). Protein concentration was determined using the bicinchoninic acid assay (Pierce,Rockford, IL, USA). 50 μg total proteins in sample buffer (62.5 mM Tris–HCl, pH 6.8, 20% glycerol, 5% β-mercaptoethanol, 0.5% bromophenol blue) were separated in SDS–PAGE electrophoresis and transferred to a nitrocellulose

membrane (Amersham Biosciences, Buckinghamshire, UK). Blotted proteins were blocked with 5% non-fat dried milk in PBS, pH 7.4, for 1h at room temperature and incubated overnight with primary antibody at a ratio of 1:500 in PBS (Diagnostic BioSystems, CA, USA). After washing three times with PBS 0.1% Tween 20, the membranes were incubated with secondary antibody–peroxidase conjugates (Amersham Biosciences) for 1h at room temperature. Protein bands were visualized using ECL detection reagents (Amersham Biosciences) in a chemisensitive visualizer (VersaDoc, BioRad, Italy).

Preliminary results

Immuno-histological assay

Elastin is a major structural component of elastic fibers that provides properties of stretch and recoil to tissues such as aorta. We have investigated the vessel wall structural modifications when in presence of human aneurysm with respect to undamaged aortas. In presence of aneurysm the vessel wall microstructure resulted deeply modified: no elastic laminae were present and elastin resulted as globular and inhomogeneous dense spots, without forming a fibrillar/laminar structure as present in the undamaged controls.

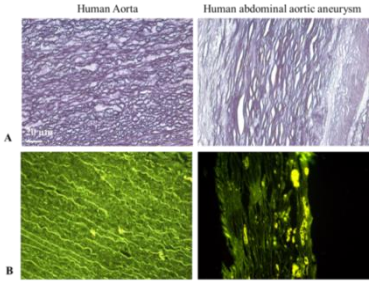

Figure1. A) Weigert's elastic stain reveals differences between elastic fibers in aorta and in abdominal aortic aneurysm. B) Immunofluorescence with elastin- antibody with FICH conjugated antibody

Immunohistochemical assay

Immunohistochemistry provides the evidences that there is not significant differences in MMP2 expression between normal human aorta (control) and aneurysm. Instead, there are significant differences between control and aneurysm for MMP9's expression.

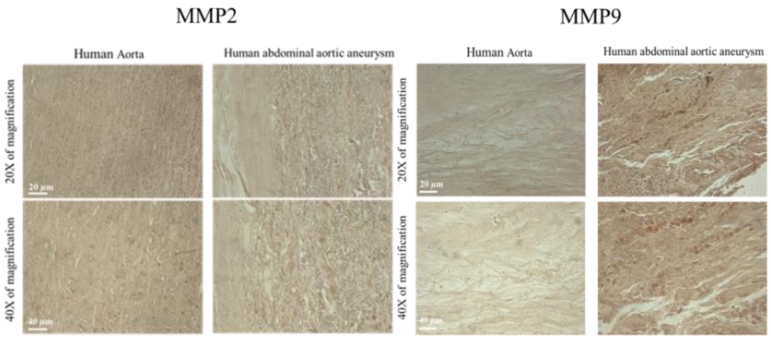

Figure2. Immunohistochemical assay for MMP2 and MMP9 expressions.

Zymography and western blot

As shown in figure 3A, MMP9 is over-expressed in human aneurysm (AAA1 and AAA2) respect to control. No differences are shown in MMP2 expression in both aneurysm and control samples. Figure 3B and 3C shown proteins involved in elastic fibers assembly. Results show no difference in elastin expression for aneurysms samples and control, while LOX expression in AAA2 seems to be down-regulated with respect to the other samples. The expression of osteopontin is over-expressed in AAA samples respect to control (figure 4D). In AAA2 sample, results show an overexpression of caspase 3 while for the AAA1 sample the expression is similar to control. Instead, caspase 9 expression in AAA1 is significantly enhanced with respect to AAA2.

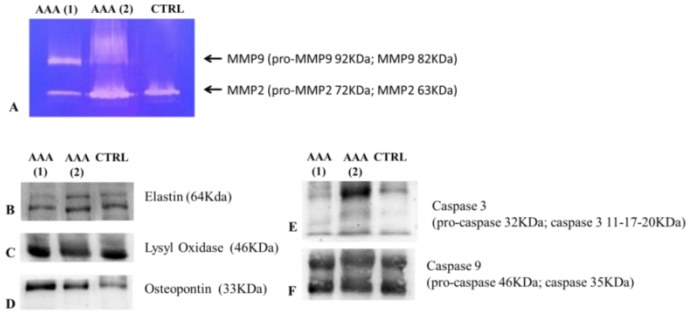

Figure 3. A) zymography for the detection of MMP2 and MMP9. B-F western blot for proteins involved in elastic fiber organization, inflammatory response and apoptosis.

Mouse model of aneurysm

We have induced aneurysm in a mouse model treated with two different concentration of $CaCl_2$ (0.25M and 0.5M). Figure 4 shows the aorta after 1 month from the surgery. In the application of both 0.25M and 0.5M concentration of $CaCl_2$, aorta becomes dilated with respect to control. However, in the second case the dilatation is more noticeable than in the first case (Figure 4A). MMP2 is over-expressed in mouse aneurysm in both concentrations of $CaCl_2$ with respect to control (PBS-treated). No differences are shown in MMP9's expression related to aneurysm and control samples (Figure 4B). As shown in western blot analysis, elastin expression is down-regulated in 0.5M concentration of $CaCl_2$, (Figure 4C).

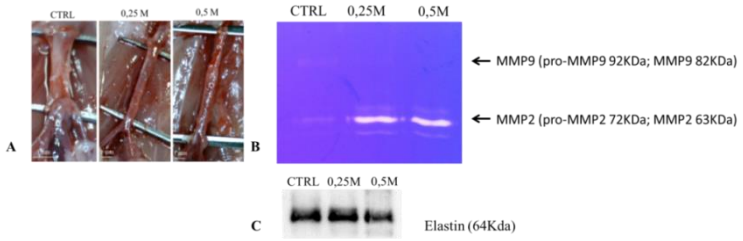

Figure 4. A) Image of mice aorta, after 1 month from the surgery. B) Analysis of MMP2 and MMP9 expression through zymography assay. C) Western blot for elastin expression.

Immuno-histological assay

The treatment of mice with $CaCl_2$ induces alteration in the vessel wall structure. AAA in mice shows marked inflammatory infiltrates including macrophages, lymphocytes in the media (Figure 5A), and the infiltration of inflammatory cells into the aortic wall involves MMPs. Elastin results destroyed in both concentration of $CaCl_2$ with respect to the control (Figure 5B). The variation between the concentration of $CaCl_2$, in inflammatory infiltrate, in elastin disaggregation, and in MMP2 over-expression is due to the increase of $CaCl_2$ applied. MMP2 analysis shows significant differences between aneurysms sample and control respect to MMP9 expression.

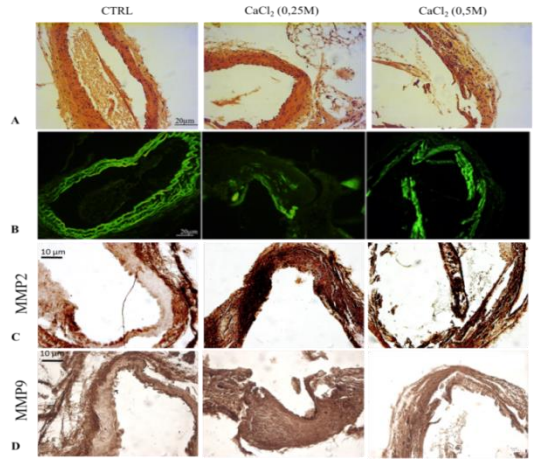

Figure 5. Imuno-histological assay.

References:

- Aimes RT, Quigley JP. Matrix metalloproteinase-2 is an interstitial collagenase. Inhibitor-free enzyme catalyzes the cleavage of collagen fibrils and soluble native type I collagen generating the specific 3/4- and 1/4-length fragments. J Biol Chem. 1995;270:5872-5876
- Allan JA, Docherty AJ, Barker PJ, Huskisson NS, Reynolds JJ, Murphy G. Binding of gelatinases A and B to type-I collagen and other matrix components. Biochem J. 1995 Jul 1;309 (Pt 1):299-306
- Becker JW, Marcy AI, Rokosz LL, Axel MG, Burbaum JJ, Fitzgerald PM, Cameron PM, Esser CK, Hagmann WK, Hermes JD, et al. Stromelysin-1: three-dimensional structure of the inhibited catalytic domain and of the C-truncated proenzyme. Protein Sci. 1995 Oct;4(10):1966-76.
- Blanchard JF, Armenian HK, Friesen PP. Risk factors for abdominal aortic aneurysm: results of a case-control study. Am J Epidemiol. 2000;151:575–583.
- Bode W, Gomis-Rüth FX, Stöckler W. Astacins, serralysins, snake venom and matrix metalloproteinases exhibit identical zinc-binding environments (HEXXHXXGXXH and Met-turn) and topologies and should be grouped into a common family, the 'metzincins'. FEBS Lett. 1993 Sep 27;331(1-2):134-40
- Bown MJ, Braund PS, Thompson J, London NJM, Samani NJ, Sayers RD. Association between the coronary artery disease risk locus on chromosome 9p21.3 and abdominal aortic aneurysm. Circ Genetics. 2008; 1:39–42.
- Bown MJ, Jones GT, Harrison SC, et al; CARDIoGRAM Consortium; Global BPgen Consortium; DIAGRAM Consortium; VRCNZ Consortium. Abdominal aortic aneurysm is associated with a variant in low-density lipoprotein receptor-related protein 1. Am J Hum Genet. 2011;89:619–627.
- Crowther M, Goodall S, Jones JL, Bell PR, Thompson MM. Localization of matrix metalloproteinase 2 within the aneurysmal and normal aortic wall. Br J Surg. 2000;87:1391-1400.
- Dhanaraj V, Ye QZ, Johnson LL, Hupe DJ, Ortwine DF, Dunbar JB Jr, Rubin JR, Pavlovsky A, Humblet C, Blundell TL. X-ray structure of a hydroxamate inhibitor complex of stromelysin catalytic domain and its comparison with members of the zinc metalloproteinase superfamily. Structure. 1996 Apr 15;4(4):375-86.

- Dorn G, Patel S, Wotherspoon G, Hemmings-Mieszczak M, Barclay J, Natt FJ, Martin P, Bevan S, Fox A, Ganju P, Wishart W, Hall J. siRNA relieves chronic neuropathic pain. Nucleic Acids Res. 2004 Mar 16;32:e49.
- Eliason JL, Hannawa KK, Ailawadi G, Sinha I, Ford JW, et al. (2005) Neutrophil depletion inhibits experimental abdominal aortic aneurysm formation. Circulation 112: 232–240.
- Elmore JR, Obmann MA, Kuivaniemi H, Tromp G, Gerhard GS, Franklin DP, Boddy AM, Carey DJ. Identification of a genetic variant associated with abdominal aortic aneurysms on chromosome 3p12.3 by genome wide association. J Vasc Surg. 2009;49:1525–1531.
- Freestone T, Turner RJ, Coady A, Higman DJ, Greenhalgh RM, Powell JT. Inflammation and matrix metalloproteinases in the enlarging abdominal aortic aneurysm. Arterioscler Thromb Vasc Biol. 1995;15:1145-1451
- Gomis-Rüth FX, Gohlke U, Betz M, Knäuper V, Murphy G, López-Otín C, Bode W. The helping hand of collagenase-3 (MMP-13): 2.7 A crystal structure of its C-terminal haemopexin-like domain. J Mol Biol. 1996 Dec 6;264(3):556-66.
- Goodall S, Crowther M, Hemingway DM, Bell PR, Thompson MM Ubiquitous elevation of matrix metalloproteinase-2 expression in the vasculature of patients with abdominal aneurysms. Circulation. 2001;104:304-309.
- Gretarsdottir S, Baas AF, Thorleifsson G, et al. Genome-wide association study identifies a sequence variant within the DAB2IP gene conferring susceptibility to abdominal aortic aneurysm. Nat Genet. 2010;42:692–697.
- Harrison SC, Smith AJP, Jones GT, et al. Interleukin-6 receptor pathways in abdominal aortic aneurysm. Eur Heart J. 2013;34:3707-3716.
- Helgadottir A, Gretarsdottir S, Thorleifsson G, et al. Apolipoprotein(a) genetic sequence variants associated with systemic atherosclerosis and coronary atherosclerotic burden but not with venous thromboembolism. J Am Coll Cardiol. 2012;60:722–729.
- Helgadottir A, Thorleifsson G, Magnusson KP, et al. The same sequence variant on 9p21 associates with myocardial infarction, abdominal aortic aneurysm and intracranial aneurysm. Nat Genet. 2008;40:217–224.
- Hinterseher I, Tromp G, Kuivaniemi H. Genes and abdominal aortic aneurysm. Ann Vasc Surg. 2011;25:388–412.
- Hong H1, Yang Y, Liu B, Cai W. Imaging of Abdominal Aortic Aneurysm: the present and the future. Curr Vasc Pharmacol. 2010;8:808-819.
- Jones GT, Bown MJ, Gretarsdottir S, et al. A sequence variant associated with sortilin-1 (SORT1) on 1p13.3 is independently associated with abdominal aortic aneurysm. Hum Mol Genet. 2013;22:2941–2947.
- Jones JA, Spinale FG, Ikonomidis JS. Transforming growth factor-beta signaling in thoracic aortic aneurysm development: a paradox in pathogenesis. J Vasc Res 2009;46:119–137.
- Lederle FA, Johnson GR, Wilson SE, Chute EP, Littooy FN, Bandyk D, Krupski WC, Barone GW, Acher CW, Ballard DJ. Prevalence and associations of abdominal aortic aneurysm detected through screening. Aneurysm Detection and Management (ADAM) Veterans Affairs Cooperative Study Group. Ann Intern Med. 1997;126:441–449.
- Lindsay ME, Dietz HC. Lessons on the pathogenesis of aneurysm from heritable conditions. Nature. 2011;473:308–316.
- Longo GM, Xiong W, Greiner TC, Zhao Y, Fiotti N, Baxter BT. Matrix metalloproteinases 2 and 9 work in concert to produce aortic aneurysms. J Clin Invest 2002;110:625–632.
- McManus MT, Sharp PA. Gene silencing in mammals by small interfering RNAs. Nat Rev Genet. 2002;3:737-747.
- Murphy G, Willenbrock F, Ward RV, Cockett MI, Eaton D, Docherty AJ. The C-terminal domain of 72 kDa gelatinase A is not required for catalysis, but is essential for membrane activation and modulates interactions with tissue inhibitors of metalloproteinases. Biochem J. 1992 May 1;283 (Pt 3):637-41.
- Nagase, H., Visse, R., Murphy, G. Structure and function of matrix metalloproteinases and timps. Cardiovascular Research 2006;69: 562-573.
- Raffetto, J.D. and Kalil, R.A. Matrix metalloproteinases and their inhibitors in vascular remodeling and vascular disease. Biochem Pharmacol 2008;75, 346-359.
- Ramanath VS, Oh JK, Sundt TM 3rd, Eagle KA. Acute aortic syndromes and thoracic aortic aneurysm. Mayo Clin Proc. 2009;84:465–481.
- Reeps C, Essler M, Pelisek J, Seidl S, Eckstein H-H, Krause B-J. Increased 18Ffluorodeoxyglucose uptake in abdominal aortic aneurysms in positron emission/computed tomography is associated with inflammation, aortic wall instability, and acute symptoms. J Vascr Surg. 2008;48:417–423.
- Sakalihasan N, Limet R, Defawe OD (2005) Abdominal aortic aneurysm. Lancet 365: 1577–1589.
- Siegel CL, Cohan RH, Korobkin M, Alpern MB, Courneya DL, et al. (1994) Abdominal aortic aneurysm morphology: CT features in patients with ruptured and nonruptured aneurysms. Am J Roentgenol 163: 1123–1129.
- Soutschek J, Akinc A, Bramlage B, et al. Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. Nature. 2004;432:173-178.
- Steffensen B[1], Wallon UM, Overall CM. Extracellular matrix binding properties of recombinant fibronectin type II-like modules of human 72-kDa gelatinase/type IV collagenase. High affinity binding to native type I collagen but not native type IV collagen. J Biol Chem. 1995 May 12;270(19):11555-66.
- Strongin AY, Collier I, Bannikov G, Marmer BL, Grant GA, Goldberg GI. Mechanism of cell surface activation of 72-kDa type IV collagenase. Isolation of the activated form of the membrane metalloprotease. J Biol Chem. 1995 Mar 10;270(10):5331-8.
- Thompson RW, Holmes DR, Mertens RA, Liao S, Botney MD, Mecham RP, Welgus HG, Parks WC. Production and localization of 92-kilodalton gelatinase in abdominal aortic aneurysms. An elastolytic metalloproteinase expressed by aneurysm-infiltrating macrophages. J Clin Invest. 1995;96:318-326.
- Van Wart HE, Birkedal-Hansen H. The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. Proc Natl Acad Sci U S A. 1990 Jul;87(14):5578-82.
- Visse R and Negase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ Res. 2003;92:827-39. Review.
- Wahlgren CM, Larsson E, Magnusson PK, Hultgren R, Swedenborg J. Genetic and environmental contributions to abdominal aortic aneurysm development in a twin population. J Vasc Surg. 2010;51:3–7; discussion 7.
- Wang Y, Krishna S., Golledge J. The calcium chloride-induced rodent model of abdominal aortic aneurysm. Atherosclerosis 226 (2013) 29e39.
- Wang HX, Yang XZ, Sun CY, Mao CQ1, Zhu YH1, Wang J. Matrix metalloproteinase 2-responsive micelle for siRNA delivery. Biomaterials. 2014 Aug;35(26):7622-34.
- Weintraub NL (2009) Understanding abdominal aortic aneurysm. N Engl J Med 361: 1114–1116.
- Xiong W, Zhao Y, Prall A, Greiner TC, Baxter BT (2004) Key roles of CD4+ T cells and IFN-c in the development of abdominal aortic aneurysms in a murine model. J Immunol 172: 2607–2612.
- Yuan SM, Jing H. Cystic medial necrosis: pathological findings and clinical implications. Rev Bras Cir Cardiovasc. 2011;26:107–115.

PUBLICATIONS:

- Arginine-glycine-glutamine and serine-isoleucine-lysine-valine-alanine-valine modified poly(l-lactide) films: bioactive molecules used for surface grafting to guide cellular contractile phenotype. Boccafoschi F, Fusaro L, Botta M, **Ramella M**, Chevallier P, Mantovani D, Cannas M. Biointerphases. 2014 Jun;9(2):029002. doi: 10.1116/1.4864432. PMID: 24985206 [PubMed - in process]
- Human elastin polypeptides improve the biomechanical properties of three-dimensional matrices through the regulation of elastogenesis. Boccafoschi F, **Ramella M**, Sibillano T, De Caro L, Giannini C, Comparelli R, Bandiera A, Cannas M. J Biomed Mater Res A. 2014 Jun 10. doi: 10.1002/jbm.a.35257. [Epub ahead of print] PMID: 24913186 [PubMed - as supplied by publisher]
- Short-term effects of microstructured surfaces: role in cell differentiation toward a contractile phenotype. Boccafoschi F, Rasponi M, **Ramella M**, Ferreira AM, Vesentini S, Cannas M. J Appl Biomater Funct Mater. 2014 Apr 18:0. doi: 10.5301/JABFM.5000186. [Epub ahead of print] PMID: 24756781 [PubMed - as supplied by publisher]
- Biological evaluation of materials for cardiovascular application: the role of the short-term inflammatory response in endothelial regeneration. Boccafoschi F, Mosca C, **Ramella M**, Carmagnola I, Chiono V, Ciardelli G, Cannas M. J Biomed Mater Res A. 2013 Nov;101(11):3131-40. doi: 10.1002/jbm.a.34630. Epub 2013 Mar 25. PMID: 23529998 [PubMed - indexed for MEDLINE]
- The effect of mechanical strain on soft (cardiovascular) and hard (bone) tissues: common pathways for different biological outcomes. Boccafoschi F, Mosca C, **Ramella M**, Valente G, Cannas M. Cell Adh Migr. 2013 Mar-Apr;7(2):165-73. doi: 10.4161/cam.23020. Epub 2013 Jan 3. Review. PMID: 23287581 [PubMed - indexed for MEDLINE] Free PMC Article

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CONGRESS

BOCCAFOSCHI F, **RAMELLA M**, FUSARO F, BOTTA M, GIANNINI C, BANDIERA A, CANNAS M (2014) Elastin-like polypeptides improve the mechanical properties of three-dimensional matrices through the regulation of elastogenesis. In: Termis Congress 2014 Tissue Engineering and regenerative medicine, Genova (Italy), 10-13 June 2014.

BOCCAFOSCHI F**,** BOTTA M, FUSARO L, **RAMELLA M** (2014). Effects of Des-Acyl Ghrelin on vascular cells. In 41st Annual ESAO Congress, Roma, 17-20 September 2014.

SEMINARS

"Molecular and cellular mechanism of tumor cachexia and its treatment". Prof. Dario Coletti.
"Epigenetic modification that control stem cell differentiation". Prof. Salvatore Oliviero.
"Developing scientific survival skills for young scientists focus on writing (quality) papers". Prof Diego Mantovani. Politecnico di Milano.
"Role of Phosphoinositides-3-kinase C2-alpha, a Class II PI 3-kinase, in development and cancer". Prof Emilio Hirsch.
"Il ruolo emergente delle vescicole extracellulari in fisiopatologia: da mediatori cellulari a biomarker" Simposio
"Assessment of cervical cancer control in Rwanda and Bhutan" Dott. Iacopo Baussano
"Ribosome alteration in cancer: effect or cause?" Prof. Fabrizio Loreni
''Atmospheric pressure plasma sources ad processes for biomedical and surface treatment applications'' Prof. Vittorio Colombo e Dr. Matteo Gherardi,
"Has nature done the experiment for us? Evolutionary insights into infection susceptibility and autoimmunity " Prof.ssa Manuela Sironi
"Disarming mutant P53 in Cancer" Prof. Gianni Del Sal
Famà Rosella "The Krüppel-like factor 2 transcription factor is a novel tumor suppressor gene recurrently mutated in Splenic Marginal Zone Lymphoma"
Ciardullo Carmela "Clonal evolution andclinical relevance of subclonal mutations in chronic lymphocyticleukemia"

LESSONS:

"Terapia Genica" . Prof.ssa Follenzi
"The C-value paradox, junk DNA and ENCODE" Dott.Cotella
"Applicazioni Terapia Genica" Prof.ssa Follenzi
The Borghese Sessions Steven R Ellis
-September 8
10:00 Clinical case – Skin as an organ
11:00 Layers of skin, cell types, developmental origins
-September 9
10:00 Cell-Cell Interactions – anchoring junctions
11:00 Cell-Cell Interactions – occluding junctions, tight junctions
-September 10
10:00 Cell Matrix Interactions – basal lamina
11:00 Epithelial-mesenchymal transition
-September 11
10:00 Angiogenesis
11:00 Innervation
-September 15
10:00 Basal layer stem cells, symmetric versus asymmetric divisions, transient amplifying cells
11:00 Solar radiation, nucleotide excision repair
-September 16
10:00 Basal and squamous cell carcinomas
11:00 Melanoma – biology
-September 17
10:00 Melanoma - treatment
11:00 Contact dermatitis
-September 22
10:00 Other skin disorders
11:00 Other components of skin