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Transcriptomic and Proteomic Profiles of Vascular Cells Involved in Human Abdominal Aortic Aneurysm

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1. Introduction

Abdominal aortic aneurysms (AAAs) are a potentially fatal disorder. Atherosclerotic changes followed by accelerated degradation of collagen and elastin, the main components of the extracellular matrix (ECM) in the vascular wall, cause the development of AAAs in deteriorating aortic walls that dilate progressively and may eventually rupture. Other alterations associated with AAAs include marked changes in the cellular composition of the aortic wall, especially the infiltration of macrophages and T-lymphocytes into the adventitia and a major reduction in the population of vascular smooth muscle cells (SMCs) (Lopez-Candales *et al.*, 1997; Henderson *et al.*, 1999).

AAA appear to be a consequence of complex mechanisms involving several potential factors: immunological, inflammatory, chemotactic, apoptotic, protease-related, angiogenic and fibrinolytic (Ailawadi *et al.*, 2003). Recently, interleukin-8 and monocyte chemoattractant protein 1 expression were shown to be raised in the AAA biopsies compared to the abdominal aorta of controls, suggesting that pathways involving these proteins may be involved in AAA pathologies (Middleton *et al.*, 2009). AAA is a disease associated with chronic inflammation in the aortic wall and leukotrienes are powerful lipid mediators released by inflammatory cells (Samuelsson, 1983). A recent study showed the increased expression of leukotriene C4 synthase together with the predominant formation of cysteinyl-leukotrienes in human AAA, linked with matrix metalloproteinases (MMP)

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production (Di Gennaro et al., 2010). This suggests a mechanism by which leukotrienes may promote matrix degradation in the AAA wall.

Despite reports that major atherosclerotic risk factors are related to AAA (Iribarren et al., 2007), we still lack the information about the process of aneurysmal degeneration (Wassef et al., 2007, Golledge et al., 2008) that we need to develop non-invasive approaches for its early detection. In aneurysm formation, collagen degradation exceeds its synthesis and together with excessive degradation of other ECM macromolecules, notably elastin, ultimately promotes rupture of these aneurysms. Increased local production of proteases, that is, enzymes capable of degrading collagen and elastin ECM proteins, have been identified in aneurysm sites (Dobrin & Mrkvicka, 1994; Knox et al., 1997; Thompson, 2006). The source of these proteases in humans is unclear, however, although many studies have measured their expression in cells from biopsies or tissues obtained after surgery (Wilson et al, 2006).

2. Purpose of the study

The aim of the study was to conduct a global comparison of the mRNA and protein expression profiles in the vascular cells to determine the genes involved in the pathological progression of aneurysmal disease by comparing the transcriptomic and proteomic profiles of SMCs and macrophages of patients with AAAs and those who have peripheral arterial occlusion (PAO) but no AAAs (verified by imaging). Using samples from two groups of patients in LILAS (the Lille Aneurysmal Study, described below), those with AAAs and others with PAO, we isolated SMCs from aortic tissue and monocytes (that were differentiated into macrophages) from blood. Although these diseases have common mechanisms, inflammatory cells essentially infiltrate the media and adventitia of the arterial wall in AAAs, whereas they are mostly in the intima in PAO. Because the factors that influence these distinctions are unknown, we designed a study that would allow us to distinguish “pro-aneurysmal” risk factors from those that are associated with atherogenesis and stenosis. We choose PAO disease as control diseases as Thompson *et al.*, (Thompson, 2002) described the separation of AAA disease from other atherosclerotic diseases like peripheral vascular disease and carotid disease.

Following recommendations for large-scale parallel quantitation of proteins (Kingsmore, 2006), we have constructed a protein microarray that contained on one slide 42 different antibodies representing proteins found by transcriptomic and proteomic analysis to be differentially modulated in macrophages and SMC from AAA and PAO patients. Finally, we performed the same analysis of plasma from the same patients to identify proteins involved in the aneurysmal process that are more easily detectable, as hypothesized that circulating biomarkers can reflect inflammation and degeneration in the AAA wall (Golledge et al., 2008).

3. Population study

The Lille Aneurysmal Study (LILAS) was a case-control study that enrolled 42 men, either AAA (case patients) or peripheral arterial occlusion (PAO) (control patients) who needed a vascular surgery or endovascular treatment at the Lille University Hospital Centre (Lille, France) (Lamblin et al., 2010). The ethics committee of the Lille University Hospital Center (France) approved the study (CCPPRB n° CP03/47 of 06 May 2003), and each patient provided written informed consent. The protocol required a blood sample (80 ml) to be taken at inclusion, before the surgery. All patients underwent the planned surgical

treatment and, when possible, surgeons removed the thrombus and took a sample of the aneurysm wall in the case patients (n=24) and a sample of the aortic wall during the bypass grafting for the control PAO patients (n=18).

The mean age of subjects was 65.6 ± 6.9 years but AAA patients were older (68.0 ± 6.1 years *versus* 62.3 ± 6.6 years). No other cardiovascular risk factors differed between the groups. In both groups, one third of the patients had hypertension and half dyslipidemia. Diabetes mellitus was found in 17% of the AAA group and 22% of the PAO group, an insignificant difference. Both groups also had similar percentages of family members with a history of heart disease (myocardial infarction and/or acute coronary syndrome): 29% in AAA patients and 35% in PAO patients. The AAA case patients (n=24) had a mean maximal external aortic diameter of 56.1 ± 11.3 [range: 46-95] mm. All the PAO patients had undergone recent vascular imaging of the aorta and lower limbs before surgery: none had AAAs or iliac, femoral or popliteal aneurysms. The groups did not differ concerning their cardiovascular treatment (Table 2, Lamblin et al, 2010). Before surgery, fewer than two thirds had antiplatelet treatment. Fewer AAA than PAO patients received ACE inhibitor treatment (21% *versus* 44%). Three of the 18 PAO patients took non-steroidal anti-inflammatory drugs and no AAA patients..

The difference of age between the two groups (AAA and PAO) of patients recruited in this study is consistent with literature on the mean age of onset of the disease in patients with similar risk factors. Also consistent with the literature is the finding that 30% of AAA patients and 50% of PAO patients have family members who have had a myocardial infarction or acute coronary syndrome.

4. Methods

4.1 Tissue biopsies from AAA and PAO patients

Aortic samples (Fig. 1) were obtained for 20 of the 24 AAA patients of the LILAS study. Because treatment procedures for PAO changed during the study period, from surgical to endovascular interventions, tissues were available for only the 4 of the 18 patients who had surgical treatment (Fig. 1).

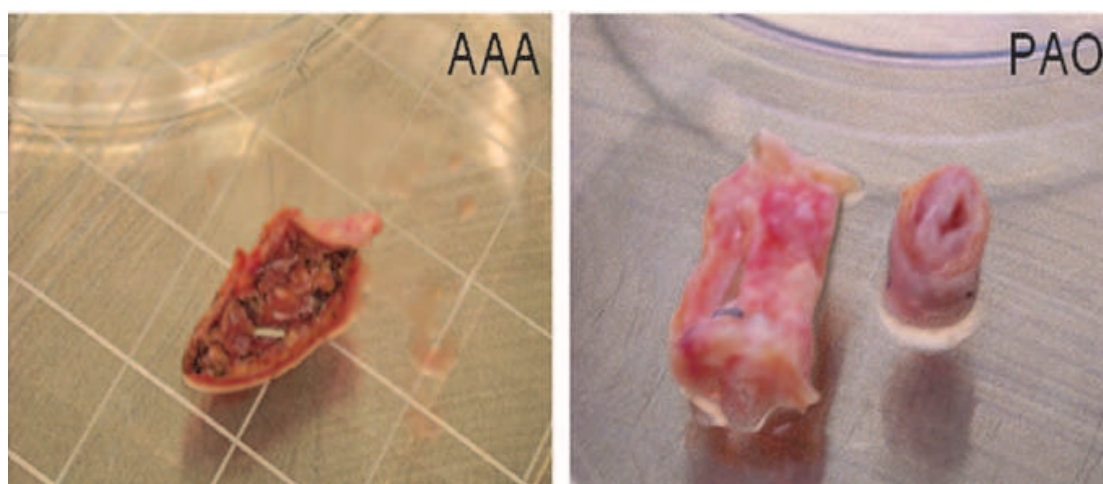


Fig. 1. Aneurysmal (AAA) and peripheral arterial occlusion (PAO) tissue used for culture of smooth muscle cells and macrophages. Example of biopsy tissue recovered for AAA (n=20) and PAO (n=4) patients.

4.2 Primary smooth muscle cells cultures

Human SMCs were obtained from all of the AAA tissue samples (n=20) and only one of the four samples from PAO patients. Individual cultures of human aortic SMCs were established for each patient by dissection of a residual segment of abdominal aorta and then cutting out the media into 1- to 2-mm³ pieces and culturing them on dishes coated with collagen type 1. Cells began to migrate from the explant after 10 days and confluence was reached in one month.

4.3 Primary cultures of human monocytes-derived macrophages

Primary cultures of human macrophages are the cell models used most often *in vitro* for human macrophages, because only a simple venous blood sample is required to investigate qualitative or quantitative macrophage modifications in a case-control study. A highly-standardized primary culture had to be devised to take into account the individual genetic variability of each blood donor, as well as the behavioral and environmental influences, and hidden abnormalities (Korke et al., 2002). Primary cultures of human macrophages were prepared as previously described (Pinet et al., 2003), with a technique adapted from Boyum (Boyum, 1968).

4.4 Transcriptomic analysis of vascular cells from AAA and PAO patients

Specific oligonucleotides for 137 genes corresponding to 24 matrix metalloproteinases (MMP), 4 tissue inhibitors of metalloproteinases (TIMP), 20 ADAMs (a disintegrin and metalloproteinase), 20 ADAMTS (ADAMS with thrombospondin motifs), 65 other proteases and 4 control genes (hydroxymethylbilane synthase, beta-actin, NADPH oxidase 1, and Glyceraldehyde-3-phosphate dehydrogenase (GADPH)) were designed with OLIGOMER software (Mediagen, France) as previously described (Lamblin et al., 2010). Data were analyzed in comparison to a reference RNA, mixture from brain, colon, heart, placenta and testis, chosen for the expression of each protease and anti-protease in at least one of these tissues. We performed a self (Cy3)-self (Cy5) hybridization of the reference RNA and observed equivalent staining throughout the microarray, except for the renin gene which we excluded from the analysis (not shown). RNA samples were extracted from human SMCs and macrophages and only RNA with a RIN value >9 was analyzed. Each sample (5 µg) was analyzed with two slides according to a dye-swap strategy to account for labeling and detection differences between Cy5 and Cy3. Data analysis was performed with the statistical language R (v 2.0.1) (Ihaka & Gentleman, 1996), more specifically with the LIMMA library (Linear Models for MicroArray data) (Smyth et al, 2003). In accordance with the MIAME (Minimum Information About a Microarray Experiment) guidelines, we note the steps involved in data processing: 1) each microarray was scanned twice (at high and low intensity) for each wavelength of Cy3 and Cy5; 2) two separate normalization steps were conducted : first, pin-by-pin and second, a lowess fitness normalization. A moderated t-statistic with empirical Bayes shrinkage of the standard error was used to classify the statistically significant modulations (Lönstedt & Speed, 2003). Because of multiple testing, p-values were corrected (Benjamini & Hochberg, 1995) to control for the false discovery rate. Genes with an adjusted p-value < 0.01 in at least three of five replications were considered further.

4.5 Proteomic analysis of vascular cells from AAA and PAO patients

Intracellular proteins from macrophages and SMCs of AAA and PAO patients were loaded (5 µg) on 2D gels. 2D-DIGE was performed as recently described (Dupont et al, 2008). Image

analysis was performed with Progenesis SameSpots v2.0 software. The differences in protein spots were then analyzed. Spots were considered to have significantly different normalized spot volumes if the fold change was greater than 1.5 and the corresponding p value (one-way ANOVA analysis) was significant. The last step applied multivariate statistics to the selected spots by calculating q values (for the false discovery rate) and power. Spots corresponding to proteins differentially expressed between the different groups of samples were identified by MALDI-TOF following the protocol previously described (Acosta-Martin et al, 2009).

4.6 Protein microarray profiling of vascular cells and plasma from AAA and PAO patients

The protein arrays allowed us to monitor 42 protein-antibody pairs and detected the profile of differentially expressed proteins in aneurysmal disease. Four differentially expressed proteins were selected from 2D analysis of macrophages from the LILAS or an earlier study: HSP70, aconitase-1, GRP75 and beta-actin (Dupont et al, 2008). Four others were selected from microarray analysis, TIMP-3, ADAMTS10, fibronectin, and tenascin. Finally, four more were identified by the 2D-analysis of plasma from patients from LILAS (personal communications), apolipoprotein AII, vitronectin, transthyretin and factor H.

5. Results

Recently, laser microdissection was used to analyze mRNA expression from macrophages and SMCs from aneurysmal tissue of a rat model of AAA created using elastase infusion (Sho et al., 2005). They found that modulation of at least 5 mRNAs differed according to cell type and flow conditions and thus concluded that a global analysis of aneurysmal tissue could mask cellular responses specific to inflammation and flow. These recent data reinforce our strategy to quantify mRNA and protein expression in two types of vascular cells from patients with different pathologies: macrophages and SMCs from AAA and PAO patients, instead of analysing the whole AAA or PAO biopsies.

5.1 Primary smooth muscle cells cultures

All the cultured human SMCs had the same elongated, spindle-shaped morphology, and at confluence all cultures assumed a hill-and-valley pattern that was maintained throughout all subcultures (Fig. 2).

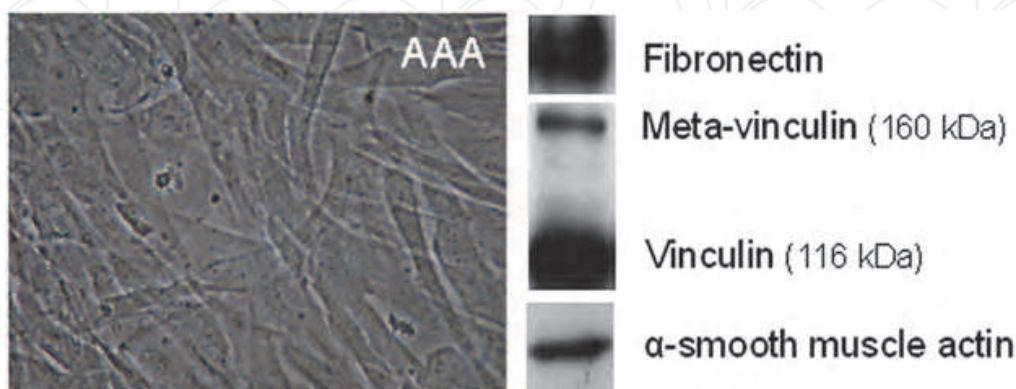


Fig. 2. Phase-contrast microscopy of smooth muscle cells obtained by explant from human AAA (n=20) and PAO (n=4) biopsies. Western blot analysis of SMC for fibronectin, meta-vinculin (160 kDa), vinculin (116 kDa) and α -smooth muscle actin.

Protein expression profiles determined by western blot analysis showed that all the cultures from both patient groups, expressed α -smooth muscle actin, vinculin and metavinculin. This finding indicates the presence of differentiated SMCs, as well as fibronectin, a component of basement membrane, that is indicative of a proliferative and secretory SMC phenotype (Fig. 2).

5.2 Primary cultures of human monocytes-derived macrophages

After, 12 days of culture, monocytes are differentiated into macrophages as shown by phase-contrast microscopy (Fig. 3).

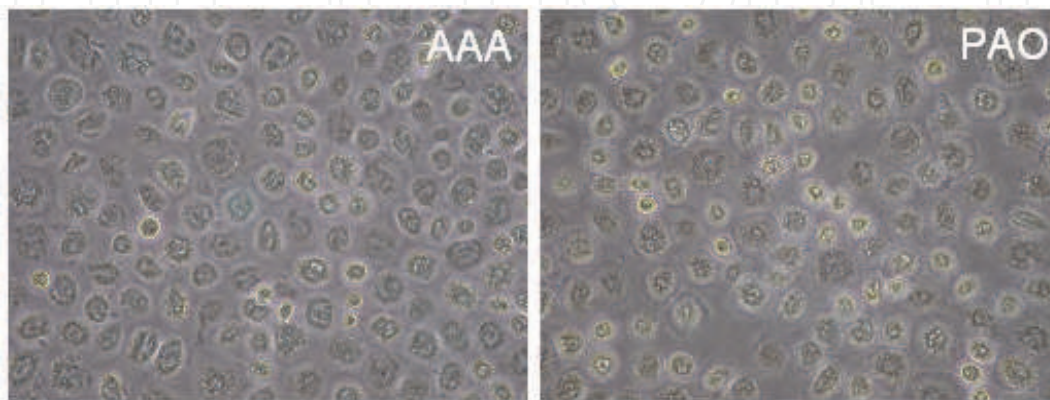


Fig. 3. Phase-contrast microscopy of macrophages prepared from blood samples obtained from human patients presenting AAA (n=16) and PAO (n=16).

The quality of macrophage cultures was evaluated by characterization of two markers, ECE-1 (Endothelin converting enzyme-1) mRNA and MMP-9 (Matrix metalloproteinase-9). ECE-1 mRNA was detected by RT-PCR on total RNA isolated from macrophages and MMP-9 activity was determined in culture medium by gelatin zymography (Pinet et al, 2003). Sixteen primary MDMs cultures, met these criteria and we observed no differences between macrophages obtained from AAA or PAO patients (Fig. 3).

5.3 Transcriptomic analysis of smooth muscle cells and macrophages from AAA and PAO patients

The importance of proteases as mediators of ECM degradation in vascular disease has been proved (Wight, 2005), as the role of other proteases and their inhibitors in AAA (Choke et al., 2005). Our global analysis on a dedicated array of proteases and anti-proteases was not limited by advance theories or hypotheses, precisely because it was intended to generate hypotheses. This technology enables more efficient selection of the possible genes involved in AAAs, as shown in two studies that used AAA specimens and a cDNA microarray (Tung et al., 2001) or quantitative RT-PCR (Higashikata et al., 2004).

We tested mRNA from SMCs of 12 different AAA patients and 2 mRNA samples from one PAO patient, and we compared them to the reference RNA. For human macrophages, mRNA expression ratio of AAA/PAO genes was tested from 4 different AAA patients and 5 different PAO patients.

The microarray analysis indicated that three mRNA sample species appeared to be significantly modulated with a q value (moderated p-value) <0.01 in SMCs from AAA compared with PAO patients: angiotensin converting enzyme (ACE), ADAMTS5 and ADAMTS8. In the AAA patients, ACE (AAA/PAO ratio: - 2.8 fold) was down-regulated,

and ADAMTS5 (AAA/PAO ratio: 1.2 fold) and ADAMTS8 (AAA/PAO ratio: 1.3 fold) were very slightly but significantly up-regulated (Fig. 4A). The graphs of individual mRNA expression (AAA/PAO ratio) for these three genes show the variability between patients (Fig. 4B). Of the 137 genes analyzed first by microarrays and then by Q-PCR for selected genes, all showed significant and concordant up- or down-regulation of expression levels for CML samples. The down-regulation of ACE mRNA in AAA patients is not surprising as a recent meta-analysis showed that ACE I/D polymorphisms are associated with a significant risk of AAAs (Thompson et al., 2008), especially in patients with hypertension (Korcz et al., 2009). Similarly, we found that ADAMTS5 and ADAMTS8 were up-regulated in SMC of AAA compared with PAO patients. These two members of the ADAMTS family are involved in vascular lesion development and can degrade different proteoglycans present in blood vessels (Yao et al., 1994).

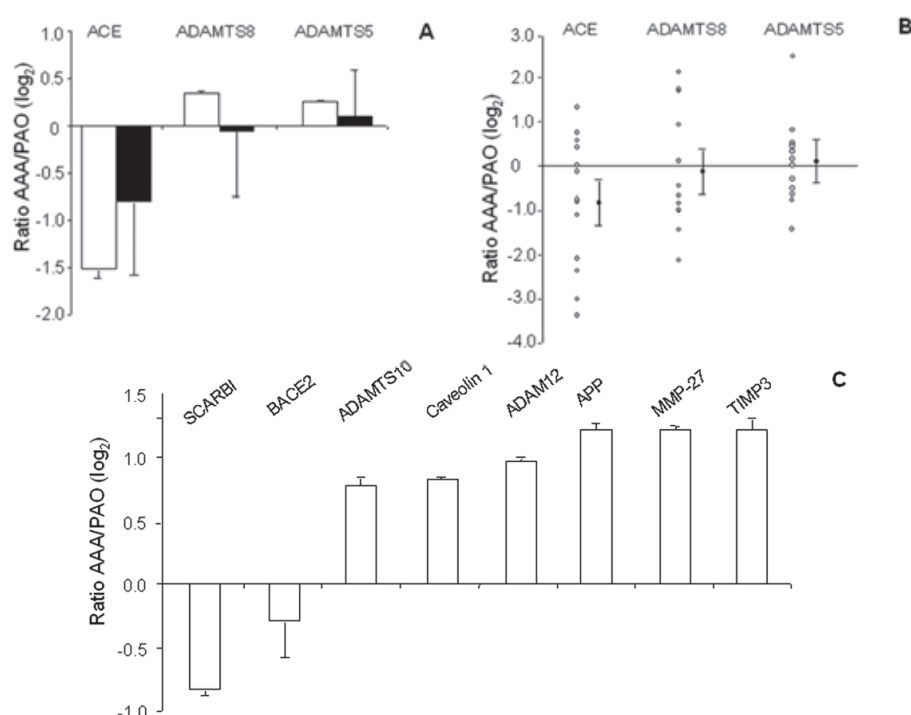


Fig. 4. mRNA expression ratio of AAA/PAO genes from human SMC and macrophages. A: mRNA expression of genes in human SMCs from AAA (n=12) and PAO (n=1) patients determined by microarray analysis (open box) and by real-time RT-PCR (grey box). B: Expression ratio as measured by real-time PCR (displayed in \log_2) of individual patients (open circles) and mean values (closed circles). The horizontal line represents an expression level identical to that of PAO ($\log_2(\text{ratio})=0$). C: mRNA expression ratio of AAA/PAO genes in human macrophages from AAA (n=4) and PAO (n=5) patients determined by microarray analysis. Data are expressed in $\log_2(\text{ratio})$.

Figure 4C shows that the mRNA samples identified as differentially expressed in macrophages from AAA and PAO patients are different from those differentially modulated in SMCs. Differential modulation was observed in eight macrophage mRNA samples. Two were down-regulated in AAA compared with PAO patients: SCARB1 (scavenger receptor class B, member 1) AAA/PAO(ratio: 1.8) and BACE2 (beta-site APP-cleaving enzyme 2) (AAA/PAO ratio: 1.2). Six were up-regulated in AAA patients: caveolin 1 (AAA/PAO ratio:

1.8), ADAMTS10 (AAA/PAO ratio: 1.7), APP (amyloid precursor protein) (AAA/PAO ratio: 2.3), MMP-27 (AAA/PAO ratio: 2.3), ADAM12 (AAA/PAO ratio: 1.9) and TIMP3 (AAA/PAO ratio: 2.3). Microarray analysis of macrophages showed the modulation of other proteases and anti-proteases, but we were unable to validate these results by RT-PCR because of the limited amount of RNA obtained from cultures. Interestingly, TIMP-3, which we found to be up-regulated in macrophages from AAA patients, has been shown to be a potent inhibitor of ADAMTS5 (Kashiwagi et al., 2001). Nonetheless, our results are consistent with the data reported (Sho et al., 2005) and thus demonstrate the interest of analyzing cell-specific compared with tissue-specific responses.

5.4 Proteomic analysis of smooth muscle cells and macrophages from AAA and PAO patients

Proteins from macrophages and SMCs of AAA and PAO patients were analysed on 2D gels. An average image was established from the scanned gels for macrophages (Fig. 5A) and for SMC (Fig. 5B) from AAA and PAO patients.

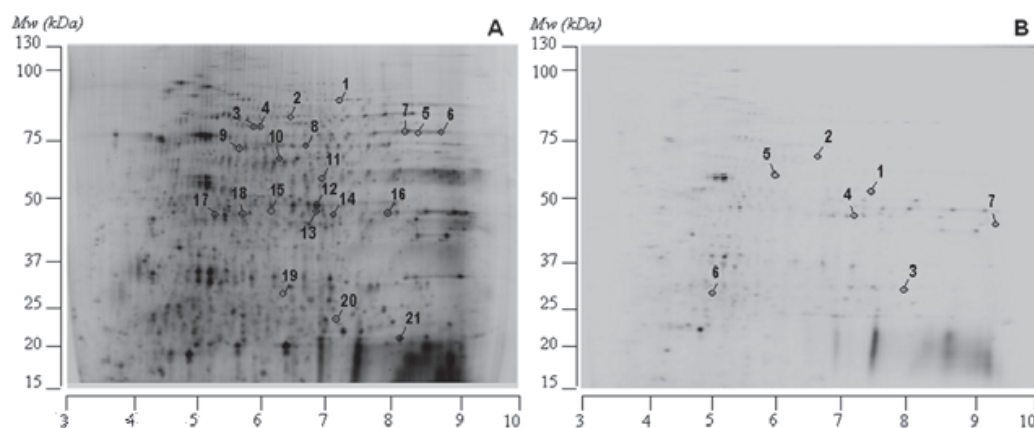


Fig. 5. Representative 2D-DIGE gel analysis of proteome of human macrophages (A) and smooth muscle cells (B) from LILAS patients. A : Macrophage proteins (5 μ g) labeled with either Cy3 or Cy5 from patients presenting AAA (n=10) or PAO (n=12) were analyzed. B : SMC proteins (5 μ g) labeled with either Cy3 or Cy5 from patients presenting AAA (n=10) or PAO (n=1) were analyzed. Gels were classified according to the presence of AAA or PAO. Polypeptidic spots differentially expressed between patients presenting AAA or PAO are indicated by a number and identified by this number in Table 1. The positions of M_r are indicated on the left and the pI on the bottom of the gels.

This differential analysis found twenty-one polypeptidic spots with differential abundance levels and a statistically reproducible difference over the series of gels. Four spots were up-regulated (spots 16, 17, 18 and 19) and 17 down-regulated (spots 1, 2, 3, 4, 5, 6, 7, 8, 9; 10, 11, 12, 13, 14, 15, 18 and 19) in macrophages from AAA compared with PAO patients (Fig. 5A). The intensity of each spot was calculated as the mean \pm SD and expressed as a percentage of normalized volume. The same approach was used for the SMC proteins, although only two 2D gels were used for the PAO SMCs (Fig. 5B). This differential analysis found seven polypeptidic spots with differential abundance levels and a statistically reproducible difference over the series of gels. Two spots were up-regulated (spots 2 and 5) and 5 down-regulated (spots 1, 3, 4, 6, and 7) in SMC from AAA compared with PAO patients.

Table 1 summarizes the identity and factor of variation of proteins differentially expressed by macrophages and SMCs from AAA and PAO patients. Of the 21 polypeptidic spots differentially expressed for macrophages, 17 were identified by mass spectrometry and corresponded to 13 non-redundant proteins. Unfortunately, we were unable to identify proteins for SMC due to either the low intensity signal on the mass spectrometry or a low probability score.

Spot number	Accession number	Protein name	Protein function	Mean of normalized value of a spot*		Fold-change ((AAA vs PAO))
				AAA	PAO	
Macrophages						
1		ND		0.005 ± 0.001	0.022 ± 0.008	0.23
2	P10636	Microtubule-associated protein tau	Promotes microtubule assembly and stability	0.008 ± 0.004	0.047 ± 0.018	0.18
3	P11142	Heat shock cognate 71 kDa protein	Chaperone	0.032 ± 0.011	0.078 ± 0.010	0.41
4		ND		0.012 ± 0.006	0.089 ± 0.025	0.13
5	P25705	ATP synthase subunit alpha	Produces ATP from ADP	0.005 ± 0.002	0.036 ± 0.010	0.14
6		Pyruvate kinase isozymes M1/M2	Glycolytic enzyme	0.029 ± 0.010	0.063 ± 0.023	0.47
7	P14618	Pyruvate kinase isozymes M1/M2	Glycolytic enzyme	0.019 ± 0.010	0.066 ± 0.024	0.29
8		ND		0.019 ± 0.005	0.090 ± 0.027	0.21
9	P30101	Protein disulfide-isomerase A3	Catalyzes the rearrangement of -S-S- bonds in proteins	0.080 ± 0.023	0.154 ± 0.055	0.52
10	P50395	Rab GDP dissociation inhibitor beta	Regulates the GDP/GTP exchange	0.072 ± 0.004	0.122 ± 0.016	0.59
11	P22695	Cytochrome b-c1 complex subunit 2	Part of the mitochondrial respiratory chain	0.036 ± 0.018	0.095 ± 0.022	0.38
12	P09467	Fructose-1,6-bisphosphatase 1	Carbohydrate biosynthesis	0.317 ± 0.077	0.771 ± 0.043	0.41
13				0.122 ± 0.023	0.284 ± 0.032	0.43
14				0.036 ± 0.013	0.073 ± 0.016	0.50
15	P40121	Macrophage-capping protein	locks the barbed ends of actin filaments	0.063 ± 0.009	0.109 ± 0.026	0.57
16	P04406	Glyceraldehyde-3-phosphate dehydrogenase	Carbohydrate degradation	0.22 ± 0.051	0.129 ± 0.025	1.71
17	P60709	Actin, cytoplasmic 1	Involved in various types of cell motility	0.266 ± 0.028	0.145 ± 0.037	1.83
18				0.266 ± 0.028	0.266 ± 0.028	0.26
18	P25774	Cathepsin S	Thiol protease	0.049 ± 0.011	0.031 ± 0.008	1.56
19	Q06830	Peroxiredoxin-1	Involved in redox regulation of the cell	0.260 ± 0.125	0.082 ± 0.011	3.18
20		ND		0.246 ± 0.05	0.386 ± 0.028	0.63

* spot number corresponds to spot indicated in Fig. 5.

Table 1. Detailed list of proteins differentially expressed between AAA and PAO samples.

Of the macrophage proteins identified, three are components of cytoskeleton: microtubule-associated protein tau (spot 2), beta-actin (spots 17 and 18) and macrophage-capping protein (spot 15) and cathepsin S (spot 18), has protease activity.

This dual transcriptomic and proteomic analysis of vascular cells from AAA and PAO patients revealed both the differences and the complementarity of microarray and 2D-electrophoresis technologies. Proteomic analysis identified only one protease, cathepsin S as differentially expressed in macrophages from AAA and PAO patients. This is consistent with previous reports showing an increase of cathepsin S activation in the wall of AAAs (Abisi et al., 2007; Abdul-Hussein et al., 2007). Still more recently, leukocyte cathepsin S was shown to be involved in macrophage apoptosis and increased SMC content and collagen deposition (De Nooijer et al., 2009). Interestingly, of the 13 proteins identified overall, three (microtubule-associated protein tau, beta-actin and macrophage-capping protein) are components of cytoskeleton, while the others are involved in protein metabolism and stress response. Unfortunately, we were unable to identify the SMC proteins corresponding to the polypeptidic spots differentially expressed.

Because of the different size of the proteome and transcriptome, we did not conduct a global correlation analysis of proteins and mRNA levels of SMCs and macrophages from these patients. Our strategy was to use specific and dedicated tools to identify the modulation of proteins and mRNAs in SMC and macrophages from AAA and PAO patients and to apply the same technology to their plasma samples.

5.5 Protein microarray profiling of SMCs, macrophages and plasma from AAA and PAO patients

Because of the limited amount of proteins available from macrophages and SMCs of our LILAS study, we used protein antibody array technology that has been shown to provide protein information in a systematic, reproducible and high-throughput fashion in several diseases (Sanchez-Carbayo et al., 2006; Weber et al., 2007).

The AAA/PAO ratio for each protein modulated is presented in detail in Table 2.

First, we analysed macrophages and SMCs proteins from LILAS patients. The microarray, revealed up-regulation in AAA patients of three macrophage proteins and down-regulation of six, as well as up-regulation of six SMC proteins and down-regulation of eight. All the proteins up-regulated in macrophages from AAA patients were also up-regulated in their SMCs: fibronectin, β -actin, tenascin. Five of six proteins down-regulated in AAA macrophages were also down-regulated in their SMCs: TIMP-3, ADAMTS5, HSP70, aconitase, GRP75.

Plasma analysis, of course, restricted the number of proteins available that could be detected as differentially modulated between the two groups. Interestingly, analysis of plasma from the LILAS patients showed only five proteins differentially expressed in plasma from AAA and PAO patients, three already selected from the macrophage and SMC samples, ADAMTS8 (AAA/PAO ratio: 0.9), ADAMTS5 (AAA/PAO ratio: 1.11) and TIMP-3 (AAA/PAO ratio: 1.15) and two only in plasma: ApoCIII (AAA/PAO ratio: 0.78) and alpha1-antitrypsin (AAA/PAO ratio: 0.88).

Of particular interest, however, is the finding that TIMP-3, ADAMTS5 and ADAMTS8 were differentially expressed in proteins from macrophages, SMCs and plasma. ADAMTS8 is known to degrade ECM proteoglycans and has the highest specific activity for cleaving aggrecan (Porter et al., 2005). Our data are in agreement with a report (Theocharis et al., 2001), which showed a decrease in the concentration of versican in human AAA. Unfortunately, tools to measure ADAMTS activity need to be developed (Wagstätter et al., 2008). Interestingly, TIMP-3 was shown to be modulated in the two cell types involved in

AAA, SMCs and macrophages and also in the plasma of the same patients, as shown with the overexpression of TIMP-3 in AAA tissue compared to aortic occlusive disease but they did not measure plasma TIMP-3 levels of the same patients as we did (Carell et al., 2002). The availability of TIMP assays allowed us to confirm the increased plasma levels of TIMP-3 in AAA patients (Lamblin et al., 2010).

Protein name	AAA	PAO	Ratio AAA/PAO
	Mean value \pm SD		
Macrophages			
TIMP-3	521 \pm 430	1409 \pm 811	0.35
ADAMTS5	439 \pm 501	1000 \pm 633	0.43
ADAMTS10	611 \pm 567	1190 \pm 596	0.45
HSP70	3634 \pm 1369	6971 \pm 2559	0.55
GRP75	4909 \pm 2331	8505 \pm 1862	0.55
ACO-1	4171 \pm 1315	7028 \pm 2958	0.60
Tenascin	27566 \pm 7751	16493 \pm 3000	1.65
Fibronectin	29278 \pm 6686	15586 \pm 3607	1.75
Beta-actin	43479 \pm 13524	23892 \pm 6088	1.80
Smooth muscle cells*			
GRP75	6393 \pm 1651	5126/8207	0.56/0.56
ADAMTS5	882 \pm 403	1479/1431	0.59/0.59
TIMP-4	1247 \pm 438	1977/2537	0.63/0.51
HSP70	7180 \pm 1700	11358/8906	0.63/0.62
Transferrin	2895 \pm 2153	4868/5076	0.67/0.59
ACO-1	6782 \pm 2088	10076/8089	0.67/0.66
ApoAII	6009 \pm 1392	8419/7622	0.71/0.63
Vitronectin	25237 \pm 2568	31398/33481	0.80/0.71
Factor H	2339 \pm 378	1733/1647	1.35/1.35
Tenascin	21849 \pm 3847	14181/13722	1.54/1.49
Beta-actin	26599 \pm 4899	16672/15917	1.59/1.56
Prealbumin	15339 \pm 1272	16175/13780	1.60/1.57
ADAMTS8	962 \pm 300	599/774	1.61/1.58
Fibronectin	23910 \pm 4485	14154/15035	1.69/1.65
Plasma			
APOCIII	14867 \pm 4892	18964 \pm 6046	0.78
Alpha1-antitrypsin	60718 \pm 6815	68871 \pm 12632	0.88
ADAMTS8	2470 \pm 523	2754 \pm 594	0.90
ADAMTS5	2176 \pm 720	1956 \pm 754	1.11
TIMP-3	2448 \pm 653	2132 \pm 610	1.15

Macrophages and SMC analysis were performed respectively from 16 and 14 AAA and 16 and 11 PAO samples; Plasma analysis was performed from 24 AAA and 18 PAO samples.* Due to the limited number of SMC samples tested, we did not calculate SD for PAO patient and gave the value from 2 independent experiments performed.

Table 2. Expression ratio of proteins in macrophages, smooth muscle cells and plasma from AAA and PAO patients.

6. Conclusion

The limitations of this study are the relatively few tissue specimens allowing study of SMCs from PAO patients, because of changes in their treatment strategy (endovascular protheses rather than surgery). To our knowledge, this is the first study to combine several global analyses to assess the changes in the expression of genes and proteins of vascular cells involved in aneurysmal disease. Its strengths include the largest and most detailed view of

changes in proteases and anti-proteases expression in patients presenting AAA. The protein array techniques confirmed that the differentially expressed proteins could also be detected in plasma. We found a restricted number of proteins differentially expressed between AAA and PAO patients: TIMP-3, ADAMTS5 and ADAMTS8 that differ significantly in plasma of AAA patients compared to PAO approach. Combining transcriptomic and proteomic is a valid approach to a better understanding of the pathophysiology of AAA but strengthen the need to investigate multiple circulating biomarkers.

7. Acknowledgment

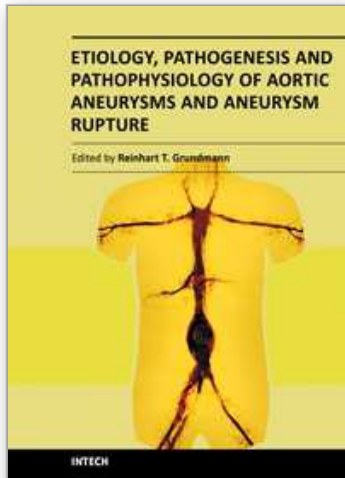
This work was supported by grants from "Fondation pour la Recherche Médicale", Région Nord Pas de Calais and a European FAD grant (Health-F2-2008-200647). All the authors declare that they have no conflicting financial interests.

8. References

- Abisi, S., Burnand, K.G., Waltham, M., Humphries, J., Taylor, P.R. & Smith, A. (2007) Cysteine protease activity in the wall of abdominal aortic aneurysms. *Journal of Vascular Surgery* 46: 1260-1266.
- Abdul-Hussein, H., Soekhoe, R.G., Weber, E., von der Thüsen, J.H., Kleemann, R., Mulder, A., van Bockel, J.H., Hanemaaijer, R. & Linderman, J.H. (2007) Collagen degradation in the abdominal aneurysm: a conspiracy of matrix metalloproteinase and cysteine collagenases. *American Journal of Pathology* 170: 809-817.
- Acosta-Martin, A.E., Chwastyniak, M., Beseme, O., Drobecq, H., Amouyel, P. & Pinet, F. (2009) Impact of incomplete DNase I treatment on human macrophage proteome analysis. *Proteomics Clinical Applications* 3: 1236-1246.
- Ailawadi, G., Eliason, J.L. & Upchurch Jr, G.R. (2003) Current concepts in the pathogenesis of abdominal aortic aneurysm. *Journal of Vascular Surgery* 38: 584-588.
- Benjamini, Y. & Hochberg, Y. (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of Royal Statistical Society* 57: 289-300.
- Boyum, A. (1968) Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. *Scandinavian Journal of Clinical Laboratory Investigation Supplement* 68: 97:9-929.
- Carrell, T.W.G., Burnand, K.G., Wells, G.M.A., Clements, J.M. & Smith, A. (2002) Stromelysin-1 (matrix metalloproteinase-3) and tissue inhibitor of metalloproteinase-3 are overexpressed in the wall of abdominal aortic aneurysms. *Circulation* 105: 477-482.
- Choke, E., Cockerill, G., Wilson, W.R.W., Sayed, S., Dawson, J., Loftus, I. & Thompson, M.M. (2005) A review of biological factors implicated in abdominal aortic aneurysm rupture. *European Journal of Vascular Endovascular Surgery* 30 : 227-244.
- De Nooijer, R., von der Thüsen, J.H., Leeuwenburgh, M.A., Overkleeft, H.S., Kraaijeveld, A.O., Dorland, R., van Santbrink, P.J., van Heiningen, S.H., Westra, M.M., Kovanen, P.T., Jukema, J.W., van der Wall, E.E., van Berke, T.J., Shi, G.P. & Biessen, E.A. (2009) Leukocyte cathepsins S is a potent regulator of both cell and matrix turnover in advanced atherosclerosis. *Arteriosclerosis Thrombosis Vascular Biology* 29: 188-194.
- Di Gennaro, A., Wagsäter, D., Mäyränpää, M.I., Gabrielsen, A., Swendenborg, J., Hamsten, A., Samuelsson, B., Eriksson, P. & Haeggström, J.Z. (2010) Increased expression of leukotriene C₄ synthase and predominant formation of cysteinyl-leukotrienes in

- human abdominal aortic aneurysm. *Proceedings National Academy of Sciences USA* 107: 21093-21097.
- Dobrin, P.B. & Mrkvicka, R. (1994) Failure of elastin or collagen as possible critical connective tissue alterations underlying aneurismal dilatation. *Cardiovascular Surgery* 2: 484-488.
- Dupont, A., Chwastyniak, M., Beseme, O., Guihot, A.L., Drobecq, H., Amouyel, P. & Pinet, F. (2008) Application of saturation dye 2D-DIGE proteomics to characterize proteins modulated by oxidized low density lipoprotein treatment of human macrophages. *Journal of Proteome Research* 7: 3572-3582.
- Golledge, J., Tsao, P.S., Dalman, R.L. & Norman, P.E. (2008) Circulating markers of abdominal aortic aneurysm presence and progression. *Circulation* 118: 2382-2392.
- Henderson, E.L., Geng, Y.J., Sukhova, G.K., Whittemore, A.D., Knox, J. & Libby, P. (1999) Death of smooth muscle cells and expression of mediators of apoptosis by T lymphocytes in human abdominal aortic aneurysms. *Circulation* 99: 96-104.
- Higashikata, T., Yamagishi, M., Sasaki, H., Minatoya, K., Ogino, H., Ishibashi-Ueda, H., Hao, H., Nagaya, N., Tomoike, H. & Sakamoto, A. (2004) Application of real-time RT-PCR to quantifying gene expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases in human abdominal aortic aneurysm. *Atherosclerosis* 177: 353-360.
- Ihaka, R. & Gentleman, R. (1996) R: a language for data analysis and graphics. *Journal of Computational Graphical Statistics* 5: 299-314.
- Iribarren, C., Darbinian, J.A., Go, A.S., Fireman, B.H., Lee, C.D. & Grey, D.P. (2007) Traditional and novel risk factors for clinically diagnosed abdominal aortic aneurysm: The Kaiser multiphasic health checkup cohort study. *Annual Epidemiology* 17: 669-678.
- Kashiwagi, M., Tortorella, M., Nagase, H. & Brew, K. (2001) TIMP-3 is a potent inhibitor of aggrecanase 1 (ADAM-TS4) and aggrecanase 2 (ADAM-TS5). *Journal of Biological Chemistry* 276: 12501-12504.
- Kingsmore, S.F. (2006) Multiplexed protein measurement: technologies and applications of protein and antibody arrays. *Nature Review Drug Discovery* 5: 310-320.
- Klein, T. & Bischoff, R. (2011) Active metalloproteases of the A disintegrin and metalloprotease (ADAM) family: biological function and structure. *Journal of Proteome Research* 10: 17-33.
- Knox, J.B., Sukhova, G.K., Whittemore, A.D. & Libby, P. (1997) Evidence for altered balance between matrix metalloproteinases and their inhibitors in human aortic diseases. *Circulation* 95: 205-212.
- Korcz, A., Mikolajczyk-Stecyna, J., Gabriel, M., Zowczak-Drabarczyk, M., Pawlaczyk, K., Kalafirov, M., Oszkinis, G. & Slomski, R. (2009) Angiotensin-converting enzyme (ACE, I/D) gene polymorphism and susceptibility to abdominal aortic aneurysm or aortoiliac occlusive disease. *Journal of Surgery Research* 153: 76-82.
- Korke, R., Rink, A., Seo, T.K., Chung, M.C., Beattle, C.W. & Hu, W.S. (2002) Genomic and proteomic perspectives in cell culture engineering. *Journal of Biotechnology* 94: 73-92.
- Lamblin, N., Ratajczak, P., Hot, D., Dubois, E., Chwastyniak, M., Beseme, O., Drobecq, H., Lemoine, Y., Koussa, M., Amouyel, P. & Pinet, F. (2010) Profile of macrophages in human abdominal aortic aneurysms: a transcriptomic, proteomic and antibody protein array study. *Journal of Proteome Research* 9: 3720-3729.
- Lönnstedt, I. & Speed, T.P. (2002) Replicated microarray data. *Statistica Sinica* 12: 31-46.
- Lopez-Candales, A., Holmes, D.R., Liao, S., Scoot, M.J., Wickline, S.A. & Thompson, R.W. (1997) Decreased vascular smooth muscle cell density in medial degeneration of human abdominal aortic aneurysms. *American Journal of Pathology* 150: 993-1007.
- Middleton, R.K., Bown, M.J., Lloyd, G.M., Jones, J.L., London, N.J. & Sayers, R.D. (2009) Characterisation of interleukin-8 and monocyte chemoattractant protein-1 expression

- within the abdominal aortic aneurysm and their association with mural inflammation. *European Journal of Vascular Endovascular Surgery* 37: 46-55.
- Pinet, F., Dupont, A., Bencherif, N., Guihot, A.L., Quatannens, B & Amouyel P. (2003) Morphology, homogeneity and functionality of human monocytes-derived macrophages. *Cellular Molecular of Biology* 49: 899-905.
- Porter, S., Clark, I.M., Kevorkian, L. & Edwards, D.R. (2005) The ADAMTS metalloproteinases. *Biochemical Journal* 386: 15-27.
- Samuelsson, B. (1983) Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation. *Science* 220: 568-575.
- Sanchez-Carbayo, M., Socci, N.D., Lozano, J.J., Haab, B.B. & Cordon-Cardo, C. (2006) Profiling bladder cancer using targeted antibody arrays. *American Journal of Pathology* 168:93-103.
- Sho, E., Sho, M., Najo, H., Kawamura, K., Masuda, H. & Dalman, R.L. (2005) Comparison of cell-type-specific vs transmural aortic gene expression in experimental aneurysms. *Journal of Vascular Surgery* 41: 844-852.
- Smyth, G.K., Yang, Y.H. & Speed, T. (2003) Statistical issues in cDNA microarray data analysis. *Methods Molecular Biology* 224: 111-136.
- Theocharis, A.D., Toslakis, I., Hjerpe, A. & Karamanos, N.K. (2001) Human abdominal aortic aneurysm is characterized by decreased versican concentration and specific downregulation of versican isoform V₀. *Atherosclerosis* 154: 367-376.
- Thompson, R.W. (2002) Reflections on the pathogenesis of abdominal aortic aneurysms. *Cardiovasc Surg* 10 :389-394.
- Thompson, R.W. (2006) Matrix metalloproteinase-8 and -9 are increased at the site of abdominal aortic aneurysm rupture. *Circulation* 113: 438-445.
- Thompson, A.R., Drenos, F., Hafez, H. & Humphries, S.E. (2008) Candidate gene association studies in abdominal aortic aneurysm: a review and meta-analysis. *European Journal of Vascular Endovascular Surgery* 35: 19-30.
- Tung, W.S., Lee, J.K. & Thompson, R.W. (2001) Simultaneous analysis of 1176 gene products in normal human aorta and abdominal aortic aneurysms using a membrane-based complementay DNA expression array. *Journal of Vascular Surgery* 34: 143-150.
- Wagstäter, D., Björk, H., Zhu, C., Björkegren, J., Valen, G., Hamsten, A. & Eriksson, P. (2008) ADAMTS-4 and -8 are inflammatory regulated enzymes expressed in macrophage-rich areas of human atherosclerotic plaques. *Atherosclerosis* 196: 514-522.
- Wassef, M., Upchurch, G.R., Kuivaniemi, H., Thompson, R.W. & Tilson III, M.D. (2007) Challenges and opportunities in abdominal aortic aneurysm research. *Journal of Vascular Surgery* 45: 192-198.
- Weber, A., Hengge, U.R., Stricker, I., Tischoff, I., Markwart, A., Anhalt, K., Dietz, A., Wittekind, C. & Tannapfel, A. (2007) Protein microarrays for the detection of biomarkers in head and neck squamous cell carcinomas. *Journal of Pathology* 38:228-238.
- Wight, T.N. (2005) The ADAMTS proteases, extracellular matrix, and vascular disease: waking the sleeping giant(s). *Arteriosclerosis Thrombosis Vascular Biology* 25: 12-14.
- Wilson, W.R., Anderton, M., Schwalbe, E.C., Jones, J.L., Furness, P.N., Bell, P.R. & Thompson, M.M. (2006) Matrix metalloproteinase-8 and -9 are increased at the site of abdominal aortic aneurysm rupture. *Circulation* 113: 438-445.
- Yao, L.Y., Moody, C., Schonherr, E., Wight, T.N. & Sandell, L.J. (1994) Identification of the proteoglycan versican in aorta and smooth muscle cells by DNA sequence anlysis, in situ hybridisation and immunohistochemistry. *Matrix Biology* 14: 213-225.



Etiology, Pathogenesis and Pathophysiology of Aortic Aneurysms and Aneurysm Rupture

Edited by Prof. Reinhart Grundmann

ISBN 978-953-307-523-5

Hard cover, 222 pages

Publisher InTech

Published online 27, July, 2011

Published in print edition July, 2011

This book considers mainly etiology, pathogenesis, and pathophysiology of aortic aneurysms (AA) and aneurysm rupture and addresses anyone engaged in treatment and prevention of AA. Multiple factors are implicated in AA pathogenesis, and are outlined here in detail by a team of specialist researchers. Initial pathological events in AA involve recruitment and infiltration of leukocytes into the aortic adventitia and media, which are associated with the production of inflammatory cytokines, chemokine, and reactive oxygen species. AA development is characterized by elastin fragmentation. As the aorta dilates due to loss of elastin and attenuation of the media, the arterial wall thickens as a result of remodeling. Collagen synthesis increases during the early stages of aneurysm formation, suggesting a repair process, but resulting in a less distensible vessel. Proteases identified in excess in AA and other aortic diseases include matrix metalloproteinases (MMPs), cathepsins, chymase and others. The elucidation of these issues will identify new targets for prophylactic and therapeutic intervention.

How to reference

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Florence Pinet, Nicolas Lamblin, Philippe Ratajczak, David Hot, Emilie Dubois, Maggy Chwastyniak, Olivia Beseme, Hervé Drobecq, Yves Lemoine, Mohammad Koussa and Philippe Amouyel (2011). Transcriptomic and proteomic profiles of vascular cells involved in human abdominal aortic aneurysm, *Etiology, Pathogenesis and Pathophysiology of Aortic Aneurysms and Aneurysm Rupture*, Prof. Reinhart Grundmann (Ed.), ISBN: 978-953-307-523-5, InTech, Available from: <http://www.intechopen.com/books/etiology-pathogenesis-and-pathophysiology-of-aortic-aneurysms-and-aneurysm-rupture/transcriptomic-and-proteomic-profiles-of-vascular-cells-involved-in-human-abdominal-aortic-aneurysm>

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