Atherosclerosis 233 (2014) 211–218

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Differential gene expression in the proximal neck of human abdominal aortic aneurysm

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ARTICLE INFO

Article history:
Received 25 November 2013
Received in revised form
16 December 2013
Accepted 22 December 2013
Available online 11 January 2014

Keywords:
Aortic aneurysm
Abdominal
Tissue array analysis
B-lymphocytes
Inflammation

ABSTRACT

Objective: Abdominal aortic aneurysm (AAA) represents a common cause of morbidity and mortality in elderly populations but the mechanisms involved in AAA formation remain incompletely understood. Previous human studies have focused on biopsies obtained from the center of the AAA however it is likely that pathological changes also occur in relatively normal appearing aorta away from the site of main dilatation. The aim of this study was to assess the gene expression profile of biopsies obtained from the neck of human AAAs.

Methods: We performed a microarray study of aortic neck specimens obtained from 14 patients with AAA and 8 control aortic specimens obtained from organ donors. Two-fold differentially expressed genes were identified with correction for multiple testing. Mechanisms represented by differentially expressed genes were identified using Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Some of the differentially expressed genes were validated by quantitative real-time PCR (qPCR) and immunohistochemistry.

Results: We identified 1047 differentially expressed genes in AAA necks. The KEGG analysis revealed marked upregulation of genes related to immunity. These pathways included cytokine–cytokine receptor interaction ($P = 8.67^{*}10^{-12}$), chemokine signaling pathway ($P = 5.76^{*}10^{-07}$), and antigen processing and presentation ($P = 4.00^{*}10^{-04}$). Examples of differentially expressed genes validated by qPCR included the T-cells marker CD44 (2.16-fold upregulated, $P = 0.008$) and the B-cells marker CD19 (3.14-fold upregulated, $P = 0.029$). The presence of B-cells in AAA necks was confirmed by immunohistochemistry.

Conclusions: The role of immunity in AAA is controversial. This study suggests that immune pathways are also upregulated within the undilated aorta proximal to an AAA.

1. Introduction

Abdominal aortic aneurysm (AAA) is a late onset degenerative condition that represents a common cause of mortality in developed countries [1]. There is currently great interest in the pathogenesis of AAA focused on identifying targets for the development of novel drug therapies to limit early stage AAA progression [2,3].

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http://dx.doi.org/10.1016/j.atherosclerosis.2013.12.017

Most studies have focused on using animal models to simulate human AAA although translation of findings from these models to patients remains uncertain [3,4]. A smaller number of studies have concentrated on assessing biopsies obtained from patients requiring open surgery to repair large, symptomatic or ruptured AAAs [5–9]. Both animal studies and investigations of end stage human AAA samples have suggested a major role of immune responses in AAA pathogenesis [5–7,10,11]. It has been suggested however that changes demonstrated within end stage human AAA biopsies may simply represent a response to the initial mechanisms driving AAA development rather than a direct cause [12].

The examination of biopsies from earlier stage human AAA could provide insight into mechanisms which are more directly involved in AAA pathogenesis. During open repair of an infra-renal AAA a clamp is usually placed directly below the renal arteries at a non-dilated part of the aorta commonly referred to as the infra-renal aortic neck [1]. Thus biopsies can be obtained from the AAA neck and histological examination of these samples demonstrates relative preservation of aortic structure with less marked damage of medial elastic fibers and mild adventitial inflammation in comparison to biopsies obtained from the body or center of the AAA [8]. Biopsies taken from the AAA neck could therefore provide insight into early mechanisms important in AAA pathogenesis. In the current study we examined human AAA neck biopsies from subjects undergoing open AAA repair. Control juxta-renal abdominal aortic samples were obtained from brain-dead heart-beating multiple organ donors [13]. We aimed to compare the whole genome expression profiles of AAA neck and control aortic biopsies in an attempt to identify mechanisms relevant to AAA.

2. Materials and methods

2.1. Patients

AAA neck specimens were obtained from a total of 26 patients undergoing open AAA repair. Full thickness AAA biopsies were obtained from the non-aneurysmal proximal neck just below the renal arteries. Specimens were collected in RNAlater® solution (Ambion) and stored at −80 °C until assayed. Juxta-renal abdominal aortic samples from 14 heart-beating brain-dead organ donors were used as controls. Aortic patches from subjects donating kidneys were obtained as previously described [13]. For AAA patients risk factors were recorded however this information was not available for organ donors. The definitions of risk factors such as dyslipidemia, hypertension, diabetes, coronary heart disease (CHD), and smoking were as previously described [14]. Dyslipidemia, hypertension, and diabetes were defined by a history of diagnosis or treatment of dyslipidemia, hypertension, or diabetes mellitus. CHD was defined by a history of myocardial infarction, angina, or treatment for coronary artery disease. Smoking was defined by history as ever or never smoker. Maximum infrarenal aortic diameter was assessed in patients with AAA from axial computed tomography angiography (CTA) images using the viewer function on a Philips workstation (MxView Visualization Workstation Software, Philips Electronics). Maximum AAA diameter was recorded in millimeters as previously reported [15,16].

2.2. Microarrays

Gene expression was initially assessed in 14 AAA neck and 8 control biopsies using microarrays. Total RNA was extracted from homogenized aortas using the TRI reagent (Sigma), and purified using RNeasy Mini Kit (Qiagen) according to manufacturer's instructions. All RNA samples were analyzed on the Agilent Bioanalyser 2100 (Agilent Technologies) for integrity using RNA Nano LabChip® (Agilent Technologies). All samples had RNA integrity number (RIN) > 7.0. Total RNA was supplied to a central facility (the Australian Genome Research Facility Ltd, Melbourne, Victoria, Australia), who conducted the entire microarray processing using Illumuna's HumanHT-12 v4 Expression BeadChip®. A total of 500 ng was labeled using the Ambion Total Prep RNA amplification kit (Cat. No. IL1791). The quantity of labeled product was ascertained using the Agilent Bioanalyser 2100 using the NanoChip protocol. A total of 1.5 μg of labeled cRNA was then prepared for hybridization to the Sentrix Human-HT12 BeadChip® by preparing a probe cocktail (cRNA at 0.05 μg/μl) that includes GEX-HYB Hybridization Buffer (supplied with the BeadChip®). A total hybridization volume of 30 μl was prepared for each sample and 30 μl loaded into a single array on the Sentrix Human-HT12 BeadChip®. The chip was hybridized at 58 °C for 16 h in an oven with a rocking platform. After hybridization, the chip was washed using the appropriate protocols as outlined in the Illumina manual. Upon completion of the washing, the chips were then coupled with Cy3 and scanned in the Illumina BeadArray Reader. The array data were converted into TXT files using BeadStudio scanner operating software. The BeadStudio file contained the summarized expression values (AVG_Signal), standard deviation of the bead replicates (BEAD_STDEV), number of beads used (Avg_NBEADS), and a detection score (Detection), which estimates the confidence limit of detection of a gene. The performance of the built-in controls that accompany each Illumina beadchip experiment was assessed as part of the BeadStudio experiment performance report and was found to be satisfactory.

2.3. Analysis of array data

The raw data matrix extracted from BeadStudio was uploaded into GeneSpring GX version 11.5.1 (Agilent Technologies Pty Ltd) software for downstream analysis. We followed the standard normalization procedures recommended for the GeneSpring GX version 11.5.1 software for Illumina single-color array. The expression values were normalized using Percentile Shift Normalization with default settings. These included normalization to 75th percentile. The expression profile of samples obtained from AAA necks was compared to those of normal aortas obtained from organ donors. We sought to identify genes with a 2-fold differential expression and corrected p value < 0.05 using the Benjamini Hochberg false discovery rate (FDR) method and determined by non-parametric Mann–Whitney U test. Genes showing $\geq$2-fold differences in expression between groups were considered to be differentially expressed. Lists of differentially expressed genes were further subjected to biological pathways analysis such as Kyoto Encyclopedia of Genes and Genomes (KEGG) using the Web-based gene set analysis toolkit (WebGestalt) software [17]. The microarray data can be obtained at the Gene Expression Omnibus (GEO) database (Series# GSE47472; http://www.ncbi.nlm.nih.gov/geo/).

2.4. Real time PCR

Quantitative real time PCR was used to assess the validity of microarray findings. Biopsies were obtained from the AAA neck of a further 12 patients and the juxta-renal aorta of a further 6 organ donors. Four genes differentially expressed in the AAA neck biopsies (IL1B, CD19, CD44, and NKTR) were chosen for further assessment. The relative expression of these four genes in each respective sample was calculated by using the concentration-Ct-standard curve method and normalized using the average expression of actin, beta (ACTB) for each sample using the Rotor-Gene Q operating software version 2.0.24. ACTB was chosen as the "housekeeping" gene since analyses showed its expression to be