

Legends of supplemental figures and tables

Figure 1: Overview of study design and results.

(A) Microarray analysis was performed on ATM and MDM isolated from 4 obese donors. After raw data gene expression filtration, 42 830 Probe sets (PSN) were selected. 7650 PSN were found to be over-expressed in ATM with fold change (FC) ≥ 1.5 and 7305 PSN were found to be lower-expressed in ATM with FC ≤ 1.5 . Cut off of Adjust p-value (p-value divided by the number of statistical tests used) at $9.99 \cdot 10^{-4}$ were chosen and allowed selection of 1371 PSN (with p-value $< 5.75 \cdot 10^{-5}$) in ATM and 1771 PSN (with p-value $< 5.27 \cdot 10^{-5}$) in MDM. Application of the Data base for Annotation Visualization and Integrative Discovery (DAVID) website identified 1066 genes as ATM highly expressed genes and used in further analysis with different softwares. (B) Gene expression after distribution and filtration, demonstrating a normal distribution in ATM and MDM as visualized by GenespringGX10. The X-axis shows normalized intensity of the raw gene expression level values. The Y-axis shows the number of genes at a given expression level.

Figure 2. Identification of genes differentially expressed between ATM and MDM. (A)

Box whisker plot representing the distribution of the normalized gene expression intensity values for the mean of ATM or MDM arrays. (B) Volcano plot illustrating FC (log base 2) compared with p-value ($-\log$ base 10) between ATM and MDM. Horizontal bar at $y = 2$ represents a significance level of $p=0.01$ (genes in grey under this line did not reach significance). Vertically only genes with a log 2 of FC >0.58 (right) and <-0.58 (left) are represented. (C) Clusters of genes higher expressed in ATM and GO categories as determined by DAVID analysis. Bars indicate the absolute number of genes belonging to each pathway. p-values are indicated. (D) GO categories of highly expressed genes in ATM as determined by ProfCom analysis. Bars indicate the percentage of genes attributed to a certain GO

category within all genes of the GO reference data set (black bars) or genes over-expressed in ATM (grey bars). Data are arranged by biological processes, cellular component and molecular function. Statistical significances of enrichment are indicated for each category.

Figure 3: Genes belonging to the CC and CXC chemokine, hematopoietins and TGFβ-related family related subfamily are highly expressed in ATM.

KEGG molecular pathways and GenespringGX10 dendrogram of CC (A) and CXC (B) chemokine family genes. The red stars indicate genes higher expressed in ATM vs MDM. Dendograms show hierarchical clustering of gene expression of CC and CXC chemokines family genes between ATM and MDM. Red indicates higher, black indicates similar and green indicates lower gene expression between ATM and MDM. (C) KEGG molecular pathways of hematopoietin gene members as depicted by DAVID (red stars indicate genes higher expressed in ATM) and GenespringGX10 dendrogram showing hierarchical clustering of the gene expression regulation of OSM, OSMR, IL-6ST between ATM and MDM. Red colour indicates higher, black indicates similar, green indicates lower gene expression between ATM and MDM.

(D) Q-PCR analysis of OSM, OSMR and IL-6ST in ATM and MDM from 7 donors. mRNA levels were normalized to cyclophilin mRNA and results expressed as mean ± SD of triplicate determinations relative to the levels in ATM set at 1.

(E) Molecular pathways of TGFβ family members as depicted by DAVID using KEGG. The red stars indicate genes higher expressed in ATM. (F) Heatmap of TGFβ and related genes in ATM and MDM. (G) Q-PCR analysis of TGFβ1, TGFβR1, TGFβ2, TGFβR2, TGFβ3, TGFβR3, TGFβ-induced, TGFβ1-induced transcript 1 (TGFβ1I1) and BMP2 expression in ATM and MDM from 7 donors. mRNA was normalized to cyclophilin mRNA and results

expressed as mean \pm SD of triplicate determinations relative to the levels in ATM set as 1. Statistically significant differences are indicated (***) $p < 0.001$. nd: non detectable

Figure 4: CXCL expression in atherosclerotic plaque inflammatory macrophages.

Q-PCR analysis of CXCL1, CXCL9, CXCL10, CXCL11 and CXCL12 expression levels performed on RNA from laser capture microdissected CD68⁺ macrophages from lipid-rich areas isolated from 5 carotid artery endarterectomy samples. mRNA levels were normalized to cyclophilin mRNA and expressed relative to the levels in ATM set at 100. Each point corresponds to a single atherosclerotic plaque sample. The median value is shown. Statistically significant differences are indicated (t-test; * $p < 0.05$, *** $p < 0.001$).

Figure 5: Prediction of ATM secreted proteins and analysis of cytokines secreted by ATM. (A) Pie-chart representing the result of *in silico* prediction of the secreted proteins in ATM-over expressed genes (1009 protein sequences were identified from 1066 genes). (B) Pie-chart showing the percentage of classically and non-classically secreted proteins. Proteins with anchor signals are indicated; proteins in the list not predicted to be secreted by the softwares are marked as unknown. (C) Representative histogram of the percentage of secreted proteins in gene subclasses according to FC values.

Figure 6: Mature adipocyte-derived lipids do not induce an ATM like-phenotype in MDM.

MDM were differentiated from human monocytes of obese subjects and treated with mature adipocytes-derived lipids (as described in Materials and Methods) or RPMI control medium for 72h. mRNA levels of CXCL1, CXCL5, CXCL10, CXCL11, CXCL12, MCP-1, TNF α , IL-1 β , IL-6 and OSM were subsequently analyzed by Q-PCR and normalized to cyclophilin

mRNA. Results are representative of 3 independent macrophage preparations and are expressed relative to the levels of cells in the presence of RPMI medium set at 1. Each bar is the mean value \pm SD of triplicate determinations. Statistically significant differences are indicated (* $p < 0.05$; *** $p < 0.001$).

Figure 7: TAM-expressed genes are enriched in ATM.

Number and percentage of Affymetrix probesets differently or equally expressed between ATM and TAM, and between MDM and TAM respectively, based on the criteria selection of a minimum cut off of 1.5-fold change (FC) in intensities per gene and a p-value $\leq 10^{-5}$. Growth factors, chemokines, proteases and other factors produced by TAM are over-expressed in ATM compared to MDM.

Table 1: Characteristics of patients used for DNA array analysis (D1-D4) and for Q-PCR validation

Donor	Age (Years)	Sex	BMI	Fasting Glucose (mmol/L)	Glycemia 2H (mmol/L)	Fasting Insulin (mUI/L)
D1	23	F	58.5	5.6	6.2	8.1
D2	27	F	53.3	5.9	8.6	12.3
D3	38	F	52.7	4.9	5.3	13.6
D4	47	F	58.7	6.6	5.1	14.4
D5	53	M	59.0	6.5	6.8	7.2
D6	54	F	68.1	7.0	8.3	12.3
D7	57	M	42.4	9.8	16.6	9.5
D8	46	F	54.9	4.5	6.6	8.1
D9	38	F	44.2	5.2	4.8	9.1
D10	43	F	43	4.9	4.3	7.7
D11	41	F	37.7	5.3	6.8	6.9

Supplemental Table 2. Primer sequences

Primer codes	Gene names	Primer sequences	
Cyclophilin	Cyclophilin	gca tac ggg tcc tgg cat ctt gtc c atg gtg atc ttc ttg ctg gtc ttg c	forward reverse
CXCL1	Chemokine (c-x-c motif) ligand 1 = GRO alpha	cca aag tgt gaa cgt gaa gtc c tct taa cta tgg ggg atg cag g	forward reverse
CXCL5	Chemokine (c-x-c motif) ligand 5 = ENA-78	ggt cct teg agc tcc ttg tg atg aac tcc ttg cgt ggt ctg	forward reverse
CXCL6	Chemokine (c-x-c motif) ligand 6 = GCP- 2	ctt gtt tac gcg tta cgc tga g ggg tcc aga caa act tgc ttc	forward reverse
CXCL9	Chemokine (c-x-c motif) ligand 9 = MIG	cca acc aag gga cta tcc acc cct tca cat ctg ctg aat ctg g	forward reverse
CXCL10	Chemokine (c-x-c motif) ligand 10 = IP-10	tgg cat tca agg agt acc tct c gca atg atc tca aca cgt gga c	forward reverse
CXCL11	Chemokine (c-x-c motif) ligand 11 = I-TAC	ggc aga tat tga gaa agc ctc c gcc ttg ctt gct tcg att tg	forward reverse
CXCL12	Chemokine (c-x-c motif) ligand 12 = SDF-1	tct caa cac tcc aaa ctg tgc c tct cca ggt act cct gaa tcc ac	forward reverse
CXCL14	Chemokine (c-x-c motif) ligand 14 = BRAK	ctg cga gga gaa gat ggt tat c gtt cca ggc gtt gta cca ctt g	forward reverse
CXCL16	Chemokine (c-x-c motif) ligand 16	ctc ctg ctg gtg tac ctg act c ccg atg gta agc tct cag gtg	forward reverse
OSM	Oncostatin M	tat agg cag ctg ctc gaa aga g ctg cag tgc tct ctc agt tta gg	forward reverse
OSMR	Oncostatin M receptor	gct tgg agc cag gaa atc ata c aat gga gct ctg aac tgg atg g	forward reverse
IL6ST	Interleukin-6 Signal transducer = gp130	gca agt ggg atc acc tat gaa g aag gag gca atg tct tcc aca c	forward reverse
TGFβ1	Transforming growth factor beta 1	ctc cga gaa gcg gta cct gaa c cac ttg cag tgt gtt atc cct	forward reverse
TGFβ2	Transforming growth factor beta 2	gac ccc aca tct cct gct aa agg cag caa tta tcc tgc ac	forward reverse
TGFβ3	Transforming growth factor beta 3	ggc tgt tga gaa gag agt cca ac cat ggt cat cct cat tgt cca c	forward reverse
TGFβR1	Transforming growth factor beta receptor 1	tat gac aac gtc agg ttc tgg c ttc ttc tcc ccg cca ctt tc	forward reverse
TGFβR2	Transforming growth factor beta receptor 2	ggg gaa aca ata ctg gct ga gag ctc ttg agg tcc ctg tg	forward reverse
TGFβR3	Transforming growth factor beta receptor 3	ggt gtg gca tct gaa gac aga g gct gtc aag gag aag ttt gct g	forward reverse
TGFβ1I1	Transforming growth factor beta Induced transcript 1	atg gcc tca ctc tct gac ttc c cag cat ggt gtc tag gct gc	forward reverse
TGFβInd	Transforming growth factor beta Induced = Big h3	ctg tgc aga agg tta ttg gca c tat cca gga cag cac tcg tag c	forward reverse
BMP2	Bone morphogenic protein 2	gca gct tcc acc atg aag aat c gag gtg ata aac tcc tcc gtg g	forward reverse
IL-6	Interleukin-6	tac ccc cag gag aag att cc ttt tct gcc agt gcc tct tt	forward reverse

TNF α	Tumor necrosis factor alpha	cag agg gcc tgt acc tca tc gga aga ccc ctc cca gat ag	forward reverse
MCP1	Monocyte chemoattractant protein-1	ccc cag tca cct gct gtt at tgg aat cct gaa ccc act tc	forward reverse
IL1 β	Interleukin-1 beta	agc tcg cca gtg aaa tga tgg cag gtc ctg gaa gga gca ctt c	forward reverse
VEGF α	Vascular endothelial growth factor α	atg gtg atg ttg gac tcc tca g cga gta cat ctt caa gcc atc c	forward reverse
FAS	Fatty acid synthase	cca ttc ccc gct gtg tcg aa aac gcc tac cag gcc atc ca	forward reverse
ICAM1	Intercellular adhesion molecule 1	ggc tgg agc tgt ttg aga ac act gtg ggg ttc aac ctc tg	forward reverse
CD36	Cluster of Differentiation 36	tca gca aat gca aag aag gga gac ggt tga cct gca gcc gtt ttg	forward reverse

Supplemental Table 3. Summary of the prediction results of ATM enriched genes coding for secreted proteins using SignalP 3.0 and SecretomP 2.0.

Class of interval of fold change (ATM vs MDM)	Number of known genes coding for proteins in each class	Number of unknown genes in each class	Number (%) of genes coding for secreted proteins found by SIGNAL P 3.0	Number (%) of genes coding for secreted proteins found by SECRETOM P 2.0	Total number (%) of genes coding for secreted proteins	Number (%) of genes coding for proteins with signal anchor found by SIGNAL P 3.0
1-100	100	0	16 (16.0 %)	23 (23 %)	39 (39 %)	6 (6.0 %)
101-200	99	1	43 (43.4 %)	16 (16.2 %)	59 (59.6 %)	5 (5.1 %)
201-300	91	9	24 (26.4 %)	11 (12.1 %)	35 (38.5 %)	5 (5.5 %)
301-400	89	11	17 (19.1%)	10 (11.0 %)	27 (30.1 %)	7 (7.9 %)
401-500	94	6	22 (23.4%)	12 (12.8 %)	34 (36.2 %)	5 (5.3 %)
501-600	94	6	13 (13.8 %)	15 (15.6 %)	28 (29.5 %)	5 (5.3 %)
601-700	94	6	15 (16.0 %)	12 (12.8 %)	27 (28.7 %)	2 (2.1 %)
701-800	97	3	6 (6.2 %)	7 (7.2 %)	13 (13.4 %)	3 (3.1 %)
801-900	91	9	11 (12.1%)	15 (16.5 %)	26 (28.6 %)	0 (0.0 %)
900-100	97	3	11.0 (11.3 %)	11.0 (11.3 %)	22 (22.7 %)	2 (2.1 %)
1001-1066	63	3	4.0 (6.3 %)	6.0 (9.5 %)	10 (15.9 %)	2 (3.2 %)
TOTAL number (%) of genes	1009.0 (94.65 %)	57.0 (5.35 %)	182 (17.07 %)	138 (12.95 %)	320 (30.02 %)	42 (3.94 %)

List 1: List of ATM highly expressed genes and results of protein secretion prediction by Signal P 3.0 and Secretom P 2.0

Results of *in silico* interrogation for secreted proteins of the highest expressed ATM genes (1066 genes with $FC \geq 1.5$ orderly ranged by classes of one hundred genes) using Signal P 3.0 and Secretome P 2.0 which identify the subset of genes with the highest probability for encoding either signal peptides (Signal P 3.0; referred to as “classical” secreted proteins) or protein sequences without signal peptide (referred to as “non classical” secreted proteins; Secretome P 2.0). For Signal P 3.0 and for Secretome P 2.0 a cut-off of 0.7 was chosen; proteins identified by an intermediary cut-off ($0.5 > \text{cut-off} < 0.7$) are marked by a star (*) and with an additional colour when predicted by both softwares (Yellow colour for Secretom P 2.0; Purple colour for Signal P 3.0), since the software instructions indicate a cut-off of 0.5 to be sufficient to predict secreted proteins. Proteins predicted to be “classical” and “non classical” secreted at the same time with a cut-off of 0.7 are referred to as “classical” secreted proteins and marked with blue colour. Proteins with membrane anchor signals in the list are indicated when predicted, whereas genes in our list not coding for proteins (or not found in Uniprot website database) are marked with dark grey colour. The 38 genes present in the cytokine antibody array belonging to the list of ATM enriched genes are indicated by light grey colour.