Gene expression in patients with abdominal aortic aneurysm - more than immunological mechanisms involved

M. Prucha[1], P. Sedivy[2], P. Stadler[2] , P. Zdrahal[2] , V. Matoska[1], H. Strnad[3]

[1] Department of Clinical Biochemistry, Hematology and Immunology, Na Homolce Hospital, Prague, Czech Republic

[2] Department of Vascular Surgery, Na Homolce Hospital, Prague, Czech Republic

[3] Institute of Molecular Genetics, Academy of Science, Prague, Czech Republic

Corresponding author: Miroslav Prucha, e-mail: miroslav.prucha@homolka.cz

Running head: gene expression in tissue of aortic abdominal aneurysm

Category: original article

Summary

Abdominal aortic aneurysm (AAA) is a serious condition of unclear pathogenesis and progression. Two samples were collected from 48 patients during AAA surgery. One sample was collected from the aneurysm, the other from the aneurysm proximal neck where the tissue did not exhibit any aneurysmal changes. Subsequently, gene expression profiles using microarrays (Illumina) were compared in RNA extracted from the samples. Overall, 2,185 genes were found to be upregulated and 2,100 downregulated; from which 158 genes had a different expression with FDR < 0.05 (False Discovery Rate) and FC ≥ 2 (Fold Change). Of this number, 115 genes were over-expressed and 43 under-expressed. The analysis of the gene list based on their biological pathways revealed that the regulation of inflammation was mediated by chemokine and cytokine signaling pathways, the integrin signaling pathway, and T and B cell activation. Moreover, a change was identified in the expression of genes involved in both intercellular and intracellular signaling systems.

Keywords: Abdominal aortic aneurysm, gene expression, immunological mechanisms, signaling systems, pathways

Introduction

Abdominal aortic aneurysm (AAA) is a serious condition with high mortality rate (Go *et al.* 2014). The mechanisms of its formation and reasons for its progression have not been clarified yet. Its pathogenesis is multifactorial and still being investigated (Golledge *et al.* 2010, Maegdefessel *et al.* 2014, Davis *et al.* 2014, Kuivaniemi *et al.* 2015). AAA is a chronic inflammatory disease characterized by inflammatory infiltration of arterial wall, degeneration and elastin fragmentation. The inflammatory process is present in the entire arterial wall and leads to extracellular matrix degradation. The only therapeutic treatment is a surgical procedure: aneurysm removal or use of a stent graft. No pharmacotherapy has been found so far for its effective treatment. Smoking is the most important risk factor for AAA. The only way known to minimize the risk of its progression and rupture is its elimination. Immunological mechanisms represent an important pathogenic factor involved in AAA development. The localized inflammatory reaction in AAA is characterized by the involvement of monocytes, macrophages, T and B lymphocytes (T and B cells) and polymorphonuclear cells. The activity of cytokines and leukotrienes, together with the dysregulation of adhesive molecules, result in the damage of smooth arterial muscle, extracellular matrix degeneration, and neovascularization (Shimizu *et al.* 2006, Rizas *et al.* 2009, Peshkova *et al*. 2016, Swedenborg *et al*. 2011, Lindholt and Shi 2006). The involvement of genetic factors in the development of the disease is indisputable (Blanchard *et al.* 2000, Sandford *et al.* 2007, Jones *et al.* 2017). Receptor mutations for TGF-beta (Transforming Growth Factor beta) and fibrillin were found in both thoracic and abdominal aortic aneurysms (Kim and Stansfield 2017). In recent years, several studies on gene expression in altered aneurysmal tissue have been published. In our opinion, no study has been carried out to compare a sample of aneurysmal and non-aneurysmal aortic tissue in the

same patient. Our study compared gene expression in the tissue of AAA and in the healthy part of the proximal neck of the aorta in the same patient.

Methods

The total of 48 patients with AAA who required an open surgical procedure were included into the study. The average diameter of all aneurysms exceeded 5.5 cm. Two samples (5 x 5 mm in size) comprising the entire thickness of the arterial wall were collected from each patient – a total of 96 samples were examined – 48 pairs of samples from 48 patients. One sample was obtained from the aneurysm bulge at the largest dilatation macroscopically, the second sample was taken from the proximal neck of the aneurysm without any macroscopically detected aneurysmal changes. Subsequently, both samples underwent Illumina microarray analysis of DNA. Baseline demographics included age and gender; data were also collected with regard to the presence of diabetes (DM), hypertension (HT), coronary heart disease (CHD) and smoking (Table 1).

Table 1 Demographic and clinical characteristics of patients

	n	m	f	Age (Mean)	DM	HT	CHD	Smoking
Patients	48	43	5	69	9	40	17	41

DM – Diabetes mellitus, HT – Hypertension, CHD – Coronary heart disease

Analysis of expression profiles

The total RNA was isolated using RNeasy Micro Kit (Qiagen, MD, USA) according to the manufacturer's protocol. Quality and concentration of RNA were measured with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, MA, USA). The RNA integrity was analyzed by the Bio-analyzer 2100 (Agilent, CA, USA). Only samples with an intact RNA profile were used for expression profiling analyses - RIN > 9 (RNA Integrity Number).

Illumina HumanHT-12 v4 Expression BeadChips (Illumina, CA, USA) were used for microarray analysis performed in accordance with the standard protocol. In brief, 200 ng RNA was amplified with Illumina TotalPrep RNA Amplification Kit (Ambion, TX, USA) and 750 ng of labeled RNA was hybridized on the chip according to the manufacturer's protocol. The analysis was performed in two biological replicates per group. The raw data were preprocessed using GenomeStudio software (version 1.9.0.24624; Illumina, CA, USA) and the Limma package (Smyth 2004) of Bioconductor (Gentleman *et al*. 2004), as described elsewhere (Valach *et al.* 2012). The transcription profiles were background-corrected using the normal-exponential model, which was quantile normalized and variance stabilized using base 2 logarithmic transformation.

Statistics

A moderated t-test was used to detect transcripts differentially expressed between the samples and controls (within Limma). False discovery rate values were used to select significantly differentially transcribed genes (false discovery rate [FDR] < 0.05). The transcription data represent minimum information about a microarray experiment (MIAME) compliant and were deposited in the ArrayExpress database. Gene set enrichment analysis and determination of gene function were performed using Enrichr web service (Chen *et al.* 2013). The Na Homolce Hospital Ethics Committee approved all protocols and informed consent forms. All patients provided informed consent for participation.

Results

Different gene expression was established in aneurysmal and non-aneurysmal tissue. Figure 1 shows heat-maps for 1,000 of the most differently expressed genes. The total of 4,285 genes with different gene expression in the aneurysmal and non-aneurysmal tissues of 11,084 transcripts were found. Of this number, 2,185 genes were over-expressed and 2,100 genes

under-expressed. Of these genes, 158 were differentially expressed with FDR < 0.05 and fold change (FC) ≥ 2. Of these, 115 were over-expressed and 43 under-expressed. The gene set enrichment analysis (GSEA) method was used to analyze the functions represented by a group of deregulated genes. The analysis performed by means of the protein analysis through evolutionary relationships (PANTHER) and Nature Pathway databases has shown changes in the signal pathways of chemokine and cytokine mediated inflammation reactions, in integrin expression, and T and B lymphocyte activation in the inflamed aneurysmal tissue. These findings were in agreement with the analysis integrated in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. In addition to immunity mechanisms, signal pathways were involved related to the regulation of inflammation, to the cytoskeleton, and signals of intercellular and intracellular pathways.

Figure 1. Heatmaps for 1,000 of the most differently expressed genes

Discussion

A number of studies have been published recently that have dealt with gene expression in AAA patients. Their summary is given in the following reviews (Estrelinha *et al*. 2014, Kuivaniemi *et al.* 2014). Gene expression was studied both in patients' plasma and/or biopsy tissue. In our study, we compared aneurysmal tissue with healthy tissue of the affected vessel, i.e. aorta of the same individual. As far as we know, it is the first study of this design. We think that the changes in gene expression manifested in the damaged tissue are more important than those detected in serum. In serum, they are associated either with a very heterogeneous mixed cell population or with an isolated cell population. With regard to the clear compartmentalization of the immune response with AAA patients, a greater importance can be supposed of the changes found directly in pathologically changed tissue similarly to hemato-oncological or oncological diseases. It is no surprise that a significant part of the changed gene expression is related to genes involved in inflammatory processes, i.e. genes for

chemokines, integrins, and those for T and B cell activation, similarly to the previously published studies (Biros *et al*. 2014, Hinterseher *et al.* 2013, Giusti *et al.* 2009, Butt *et al.* 2016, Tanios *et al.* 2015). Destructive inflammation is the main characteristic of AAA formation and progression; it is therefore understandable that immunological mechanisms are at play. The results show that both B and T lymphocytes (i.e. both cell and humoral immunity) are involved. Regarding the basic pathogenetical mechanism (chronic inflammation), it was not surprising, e.g. activation of B lymphocytes "promotes AAA by producing immunoglobulins, cytokines and metalloproteinases resulting in the activation of macrophages, mast cells and complement pathways (Zhang *et al.* 2015). Similarly, our study demonstrated the activation of the pathways regarding the chemokine signaling, cytokine receptor interaction, leukocyte transendothelial migration, and Toll like receptor signaling. The selected pathways and differentially expressed genes discussed in the following text are listed in Table 2. When interpreting our results, we focused on the pathways associated with differently expressed genes. When analyzing associated signal pathways in 158 genes with the most significant differences in expression, we found 17 inhibited pathways and 27 activated pathways. Many of these pathways are associated with inter and intracellular signaling. Inter and intra-cellular signaling plays an essential role for the correct function of the cells and thus also tissues (Antebi e*t al*.2017). What we consider to be important is the evaluation of the associated pathways and trends of the changes found: whether the activation or inhibition is going on. The detection of activated associated signal pathways is the most common trend. Speaking of individual signal pathways, it is interesting to mention a different expression of genes that are associated with dilated cardiomyopathy (DCM). The pathway of dilated cardiomyopathy with differentially expressed genes are on Figure 2. These findings suggest that the two diseases are related. DCM is a disease that is mostly autosomal dominant and more rarely displaying autosomal recessive heredity or X-linked inheritance (Mestroni *et al*.

2014). More than 40 genes related to DCM (Human Gene Mutation Database) have been detected so far (Tayal *et al.* 2017). When the genes involved in the signal pathway for DCM were activated, a reduced expression of genes associated with intracellular signaling in aneurysmal tissue was found. Inhibition of the pathways associated with the regulation of cytoskeleton shows a potential insufficient function of the mechanisms modulating actin cytoskeleton, which is a very important mechanism in cardiovascular diseases (Tang and Gerlach 2017). The other example of changes related to intercellular communications is the pathway of Gap junctions (Figure 3). Gap junctions (GJs) represent an intercellular network of protein channels that facilitate the cell-to-cell passage of ions, hormones and neurotransmitters. These intercellular channels comprise a protein family known as connexins. GJs are surface membrane structures that allow direct communication between cells. They were discovered in the 1960s following the convergence of the detection of low-resistance electrical interactions between cells and anatomical studies of intercellular contact points (Evans 2015). GJs are significantly involved in controlling vascular functions (Figueroa and Duling 2009). These findings correspond to the study by Lenk et al,, who also proved a significant difference in expression genes associated with GAP and other signaling pathways (Lenk *et al.* 2007). The expression of vascular connexins is altered in hypertension. Since our AAA group of patients was characterized by a high incidence of hypertension, it would be interesting to know whether this change is associated with AAA or hypertension. The phosphatidylinositol signaling system is another intracellular signaling system with inhibited expression (Figure 4). This system regulates various structural and developmental functions but is also centrally involved in a plethora of signal transduction pathways in all eukaryotic models. They are not only precursors of second messengers but also directly interact with many protein effectors, thus regulating their localization and/or activity (Delage *et al*. 2014). The phosphatidylinositol signaling system is an important lipid signaling system (Wymann

and Schneiter 2008). Its altered function in AAA may provide potential evidence of its involvement in AAA pathogenesis. When interpreting the inhibited expression of the above-mentioned signaling systems, we can generalize that what we see is a defect in intracellular communication with all its consequences leading to poor cell functioning. We may only speculate about the causes of these changes, which will no doubt be the subject of further studies. One of the interesting outcomes of our study is the identification of “local” pathology in relation to a different gene expression. A number of questions arise in this connection. It is known from the clinical practice that there is practically no recurrence with the patients after the operation. Why would the pathology only be limited to a specific section of aorta and significantly depends on the age of the patient? From the existing studies it follows that aneurysm of chest aorta has significantly lower prevalence and that gene expression TAA differs from gene expression AAA(Matsumoto *et al.* 2014). It might be caused by a different embryonic origin of chest and abdominal aortas, which leads to a different response of the tissue, e.g. to cytokines.

Limitations of the study. We cannot be sure that the sample obtained from the neck of aneurysm, represented a healthy vessel tissue. Nevertheless, the heatmaps of the analysis performed provide good evidence for the homogeneity of the samples collected from the neck of aneurysm and that were assessed by a surgeon as “normal” tissue. We did not perform protein analysis of the selected genes that were identified as the most significantly differently expressed. Such a selection, however, would have been problematic owing to their high number. Taking samples from the same patient represents a possible limitation of the study if the genetic factors played a significant role in the onset of the disease. While in the thoracic aortic aneurysms (TTA), we already know a specific list of genes that are undoubtedly associated with the pathogenesis of TAA, no mutation predicting the occurrence of AAA has yet been found. (Carino D et al., 2018). Therefore, it will certainly not be a monogenic

disease. The results of recent meta-analysis have shown only a limited number of AAA-associated nucleotide polymorphisms in the studies performed so far (Bradley et al., 2016). The other limitation is the limited number of patients resulted from the financial costs of the study.

In summary, differences in gene expression in the aneurysmal tissue and in the biopsy of healthy tissue of the same patient were found. The changes detected were related to the inflammatory process regulation by means of immune mechanisms - inflammation mediated by chemokine and cytokine signaling pathways, integrin signaling pathway, T and B cell activation. Besides, changes in the expression of genes involved in intercellular and intracellular signaling systems were identified. Knowledge of these mechanisms can help get better understanding of the pathogenesis and treatment of AAAs.

Acknowledgements

This study was supported by an MH CZ - DRO (Hospital Na Homolce – NNH, 00023884), IG144101. Technical support was kindly provided by Petr Sikl.

Conflict of interest

None.

References

1. ANTEBI YE, NANDAGOPAL N, ELOWITZ MB: An operational view of intercellular signaling pathways. *Curr Opin Syst Biol* **1**:16-24, 2017.
2. BIROS E, MORAN CS, RUSH CM, GÄBEL G, SCHREURS C, LINDEMAN JH, WALKER PJ, NATAATMADJA M, WEST M, HOLDT LM, HINTERSEHER I,

PILARSKY C, GOLLEDGE J: Differential gene expression in the proximal neck of human abdominal aortic aneurysm. *Atherosclerosis* **233**: 211–218, 2014.

3. BLANCHARD JF, ARMENIAN HK, FRIESEN PP: Risk factors for abdominal aortic aneurysm: results of a case-control study. *Am J Epidemiol* **151**: 575–583, 2000.
4. BRADLEY DT, BADGER SA, McFARLAND M, HUGHES AE: Abdominal aortic aneurysm genetic associations: mostly false? A systematic review and meta analysis. Eur J Vasc Endovasc Surg 51: 64-75, 2016
5. BUTT HZ, SYLVIUS N, SALEM MK, WILD JB, DATTANI N, SAYERS RD, BOWN MJ: Microarray-based gene expression profiling of abdominal aortic aneurysm. *Eur J Vasc Endovasc Surg* **52**: 47–55, 2016.
6. CARINO D, SARAC TP, ZIGANSHIN BA, ELEFTERIADES JA: Abdominal aortic aneurysm: Evolving controversies and uncertainties. Int J Angiol **27**: 58-80, 2018.
7. CHEN EY1, TAN CM, KOU Y, DUAN Q, WANG Z, MEIRELLES GV, CLARK NR, MA'AYAN A: Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC Bioinformatics* **14:** 128, 2013.
8. DAVIS FM, RATERI DL, DAUGHERTY L: Mechanisms of aortic aneurysm formation: translating preclinical studies into clinical therapies. *Heart* **100**: 1498–1505, 2014.
9. DELAGE E, PUYAUBERT J, ZACHWSKI A, RUELLAND E: Signal transduction pathways involving phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate: convergences and divergences among eukaryotic kingdoms. *Prog Lipid Res* **52**: 1–14, 2013.
10. ESTRELINHA M, HINTERSEHER I, KUIVANIEMI H: Gene expression studies in human abdominal aortic aneurysm. *Reviews in Vascular Medicine* **3**:77-82, 2014.

11. EVANS WH: Cell communications across gap junctions: a historical perspective and current developments. *Biochem Soc Trans* **43**: 450–459, 2015.

12. FIGUEROA XF, DULING BR: Gap junctions in the control of vascular function. *Antioxidants Redox Signaling* **11**: 251–265, 2009.

13. GENTLEMAN RC1, CAREY VJ, BATES DM, BOLSTAD B, DETTLING M, DUDOIT S, ELLIS B, GAUTIER L, GE Y, GENTRY J, HORNIK K, HOTHORN T, HUBER W, IACUS S, IRIZARRY R, LEISCH F, LI C, MAECHLER M, ROSSINI AJ, SAWITZKI G, SMITH C, SMYTH G, TIERNEY L, YANG JY, ZHANG J: Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol* **5**: R80, 2004.

14. GIUSTI B, ROSSI L, LAPINI I, MAGI A, PRATESI G, LAVITRANO M, BIASI GM, PULLI R, PRATESI C, ABBATE R: Gene expression profiling of peripheral blood in patients with abdominal aortic aneurysm. *Eur J Vasc Endovasc Surg* **38**: 104–112, 2009.

15. GO AS, MOZAFFARIAN D, ROGER VL, BENJAMIN EJ, BERRY JD, BLAHA MJ, DAI S, FORD ES, FOX CS, FRANCO S, FULLERTON HJ, GILLESPIE C, HAILPERN SM, HEIT JA, HOWARD VJ, HUFFMAN MD, JUDD SE, KISSELA BM, KITTNER SJ, LACKLAND DT, LICHTMAN JH, LISABETH LD, MACKEY RH, MAGID DJ, MARCUS GM, MARELLI A, MATCHAR DB, MCGUIRE DK, MOHLER ER 3RD, MOY CS, MUSSOLINO ME, NEUMAR RW, NICHOL G, PANDEY DK, PAYNTER NP, REEVES MJ, SORLIE PD, STEIN J, TOWFIGHI A, TURAN TN, VIRANI SS, WONG ND, WOO D, TURNER MB; AMERICAN HEART ASSOCIATION STATISTICS COMMITTEE AND STROKE STATISTICS SUBCOMMITTE. Executive summary: heart disease and stroke statistics—2014

update: a report from the American Heart Association: *Circulation* **129**: 399–410, 2014.

16. GOLLEDGE J, NORMAN PE: Atherosclerosis and abdominal aortic aneurysm. Cause, response, or common risk factors? *Arterioscl Thromb Vasc Biol* **30**: 1075–1077, 2010.
17. HINTERSEHER I1, ERDMAN R, ELMORE JR, STAHL E, PAHL MC, DERR K, GOLDEN A, LILLVIS JH, CINDRIC MC, JACKSON K, BOWEN WD, SCHWORER CM, CHERNOUSOV MA, FRANKLIN DP, GRAY JL, GARVIN RP, GATALICA Z, CAREY DJ, TROMP G, KUIVANIEMI H: Novel pathways in the pathobiology of human abdominal aortic aneurysms. *Pathobiology* **80:** 1–10, 2013.
18. JONES GT, TROMP G, KUIVANIEMI H, GRETARSDOTTIR S, BAAS AF, GIUSTI B, STRAUSS E, VAN'T HOF FN, WEBB TR, ERDMAN R, RITCHIE MD, ELMORE JR, VERMA A, PENDERGRASS S, KULLO IJ, YE Z, PEISSIG PL, GOTTESMAN O, VERMA SS, MALINOWSKI J, RASMUSSEN-TORVIK LJ, BORTHWICK KM, SMELSER DT, CROSSLIN DR, DE ANDRADE M, RYER EJ, MCCARTY CA, BÖTTINGER EP, PACHECO JA, CRAWFORD DC, CARRELL DS, GERHARD GS, FRANKLIN DP, CAREY DJ, PHILLIPS VL, WILLIAMS MJ, WEI W, BLAIR R, HILL AA, VASUDEVAN TM, LEWIS DR, THOMSON IA, KRYSA J, HILL GB, ROAKE J, MERRIMAN TR, OSZKINIS G, GALORA S, SARACINI C, ABBATE R, PULLI R, PRATESI C, SARATZIS A, VERISSIMO AR, BUMPSTEAD S, BADGER SA, CLOUGH RE, COCKERILL G, HAFEZ H, SCOTT DJ, FUTERS TS, ROMAINE SP, BRIDGE K, GRIFFIN KJ, BAILEY MA, SMITH A, THOMPSON MM, VAN BOCKXMEER FM, MATTHIASSON SE, THORLEIFSSON G, THORSTEINSDOTTIR U, BLANKENSTEIJN JD, TEIJINK JA, WIJMENGA C, DE GRAAF J, KIEMENEY LA, LINDHOLT JS, HUGHES A,

BRADLEY DT, STIRRUPS K, GOLLEDGE J, NORMAN PE, POWELL JT, HUMPHRIES SE, HAMBY SE, GOODALL AH, NELSON CP, SAKALIHASAN N, COURTOIS A, FERRELL RE, ERIKSSON P, FOLKERSEN L, FRANCO-CERECEDA A, EICHER JD, JOHNSON AD, BETSHOLTZ C, RUUSALEPP A, FRANZÉN O, SCHADT EE, BJÖRKEGREN JL, LIPOVICH L, DROLET AM, VERHOEVEN EL, ZEEBREGTS CJ, GEELKERKEN RH, VAN SAMBEEK MR, VAN STERKENBURG SM, DE VRIES JP, STEFANSSON K, THOMPSON JR, DE BAKKER PI, DELOUKAS P, SAYERS RD, HARRISON SC, VAN RIJ AM, SAMANI NJ, BOWN MJ: Meta-Analysis of Genome-Wide Association Studies for Abdominal Aortic Aneurysm Identifies Four New Disease-Specific Risk Loci. *Circ Res* **120**:341-353, 2017.

19. KIM HW, STANSFIELD BK: Genetic and epigenetic regulation of aortic aneurysms. *Biomed Res Int* **2017**: 7268521, 2017.
20. KUIVANIEMI H, RYER EJ, ELMORE JR, TROMP G: Understanding the pathogenesis of abdominal aortic aneurysms. *Expert Rev Cardiovasc Ther* **13:** 975-987, 2015.
21. LENK GM, TROMP G, WEINSHEIMMER S, GATALICA Z, BERGUER R, KUIVANIEMI H. Whole genome expression profiling reveals a significant role for immune function in human abdominal aortic aneurysms. *BMC Genomics* **8**:237, 2007.
22. LINDHOLT J, SHI GP: Chronic inflammation, immune response, and infection in abdominal aortic aneurysms. *Eur J Vasc Endovasc Surg* **31**: 453–463, 2006.
23. LU S1, WHITE JV, LIN WL, ZHANG X, SOLOMIDES C, EVANS K, NTAOULA N, NWANESHIUDU I, GAUGHAN J, MONOS DS, OLESZAK EL, PLATSOUCAS CD: Aneurysmal lesions of patients with clonally expanded T cells abdominal aortic aneurysm contain clonally expanded T cells. *J Immunol* **192**: 4897–4912, 2014.

24. MAEGDEFESSEL L, DALMAN RL, TSAO PS: Pathogenesis of abdominal aortic aneurysms: microRNAs, proteases, genetic associations. *Annu Rev Med* **65**: 49–62, 2014.

MATSUMOTO KI, SATAH K, MANIWA T, TANAKA T, OKUNISHI H, ODA T: Proteomic comparison between abdominal and thoracic aortic aneurysms. *Internat J Mol Med* **33**:1033-1047, 2014.

25. MESTRONI L, BRUN F, SPEZZACATENE A, SINAGRA G, TAYLOR MR: Genetic causes of dilated cardiomyopathy. *Porg Pediatr Cardiol* **37:** 13–18, 2014.

26. PESHKOVA JO, SCHAEFER G, KOLTSOVA EK: Atherosclerosis and aortic aneurysm – is inflammation a common denominator? *FEBS* **283**: 1636–1652, 2016.

27. RIZAS KD, IPPAGUNTA N, TILSON MD 3RD: Immune cells and molecular mediators in the pathogenesis of the abdominal aortic aneurysm. *Cardiol Rev* **17:** 201–210, 2009.

28. SANDFORD RM, BOWN MJ, LONDON NJ, SAYERS RD: The genetic basis of abdominal aortic aneurysms: a review. *Eur J Vasc Endovasc Surg* **33**: 381-390, 2007.

29. SHIMIZU K, MITCHELL RN, LIBBY P: Inflammation and cellular immune responses in abdominal aortic aneurysms. *Arterioscler Thromb Vasc Biol* **26**: 987–994, 2006.

30. Smyth GK. Linear models and empirical bayes methods for assessing differential expression in microarray experiments. *Stat Appl Genet Mol Biol* **3**: Article 3, 2003.

31. SWEDENBORG J, MÄYRÄNPÄÄ MI, KOVANEN PT: Mast cells: important players in the orchestrated pathogenesis of abdominal aortic aneurysms. *Arterioscl Thromb Vasc Biol* **31:** 734–740, 2011.

32. TANG DD, GERLACH BD. The role and regulation of the actin cytoskeleton, intermediate filaments and microtubules in smooth muscle cells migration. *Respiratory Research* **18**:54-66, 2017.

33. TANIOS F, PELISEK J, LUTZ B, REUTERSBERG B, MATEVOSSIAN E, SCHWAMBORN K, HÖSEL V, ECKSTEIN HH, REEPS C: XC4: a potential marker for inflammatory activity in abdominal aortic aneurysm wall. *Eur J Vasc Endovasc Surg* **50**: 745–753, 2015.

34. TAYAL U, PRASAD S, COOK SA: Genetics and genomics of dilated cardiomyopathy and systolic heart failure. *Genome Medicine* **9:** 20–34, 2017.

35. VALACH J1, FÍK Z, STRNAD H, CHOVANEC M, PLZÁK J, CADA Z, SZABO P, SÁCHOVÁ J, HROUDOVÁ M, URBANOVÁ M, STEFFL M, PAČES J, MAZÁNEK J, VLČEK C, BETKA J, KALTNER H, ANDRÉ S, GABIUS HJ, KODET R, SMETANA K JR, GÁL P, KOLÁŘ M: Smooth muscle actin-expressing stromal fibroblasts in head and neck squamous cell carcinoma: increased expression of galectin-1 and induction of poor prognosis factors. *Int J Cancer* **131**: 2499–2508, 2012.

36. WYMANN MP, SCHNEITER R: Lipid signalling in disease. *Nature Rev Mol Cell Biology* **9**: 162–176, 2008.

37. ZHANG L, WANG Y: B lymphocytes in abdominal aortic aneurysms. *Atheriosclerosis* **242**: 311-317, 2015.

Figure 1

Heat-maps for 1.000 of the most differently expressed genes

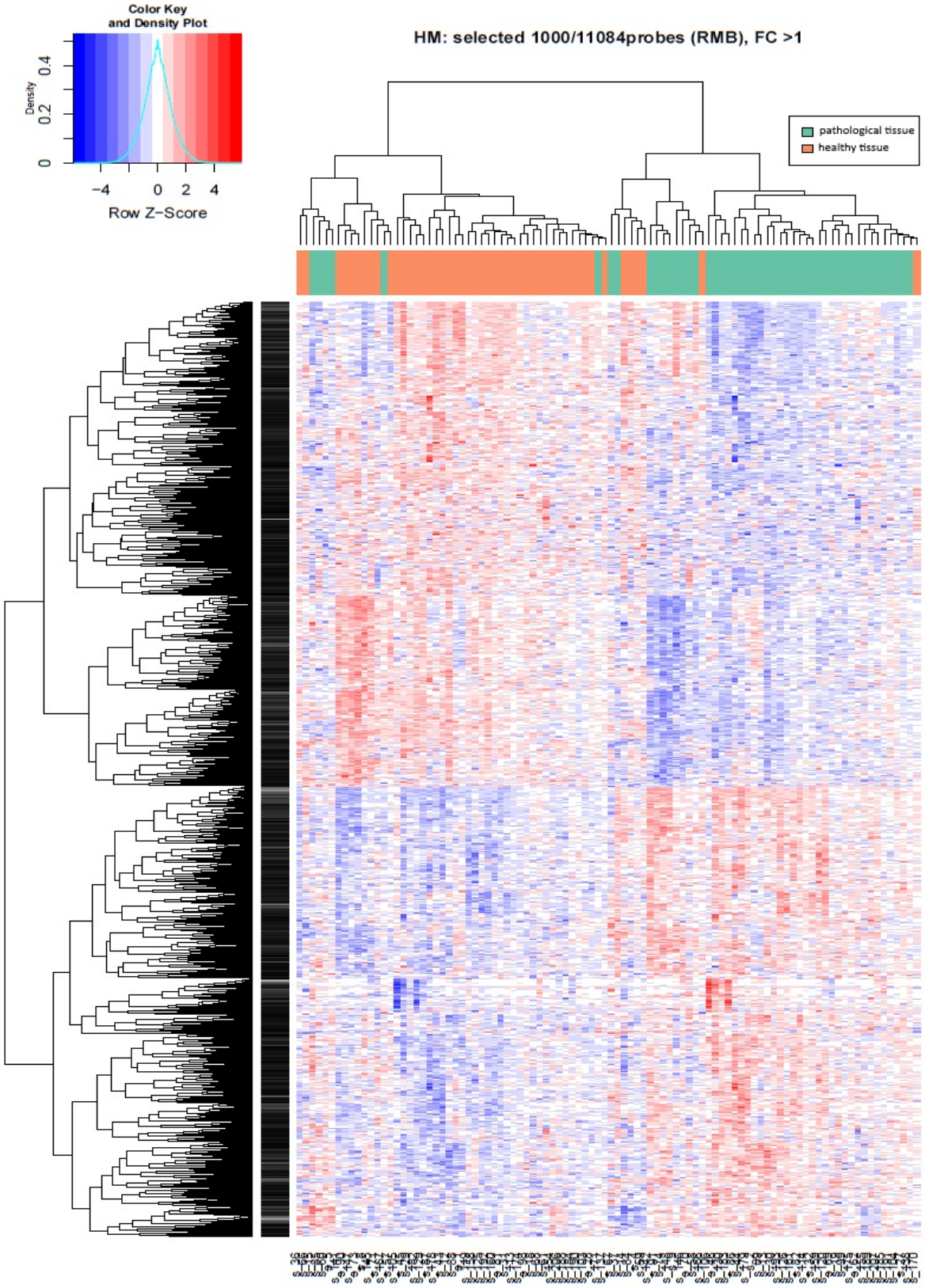


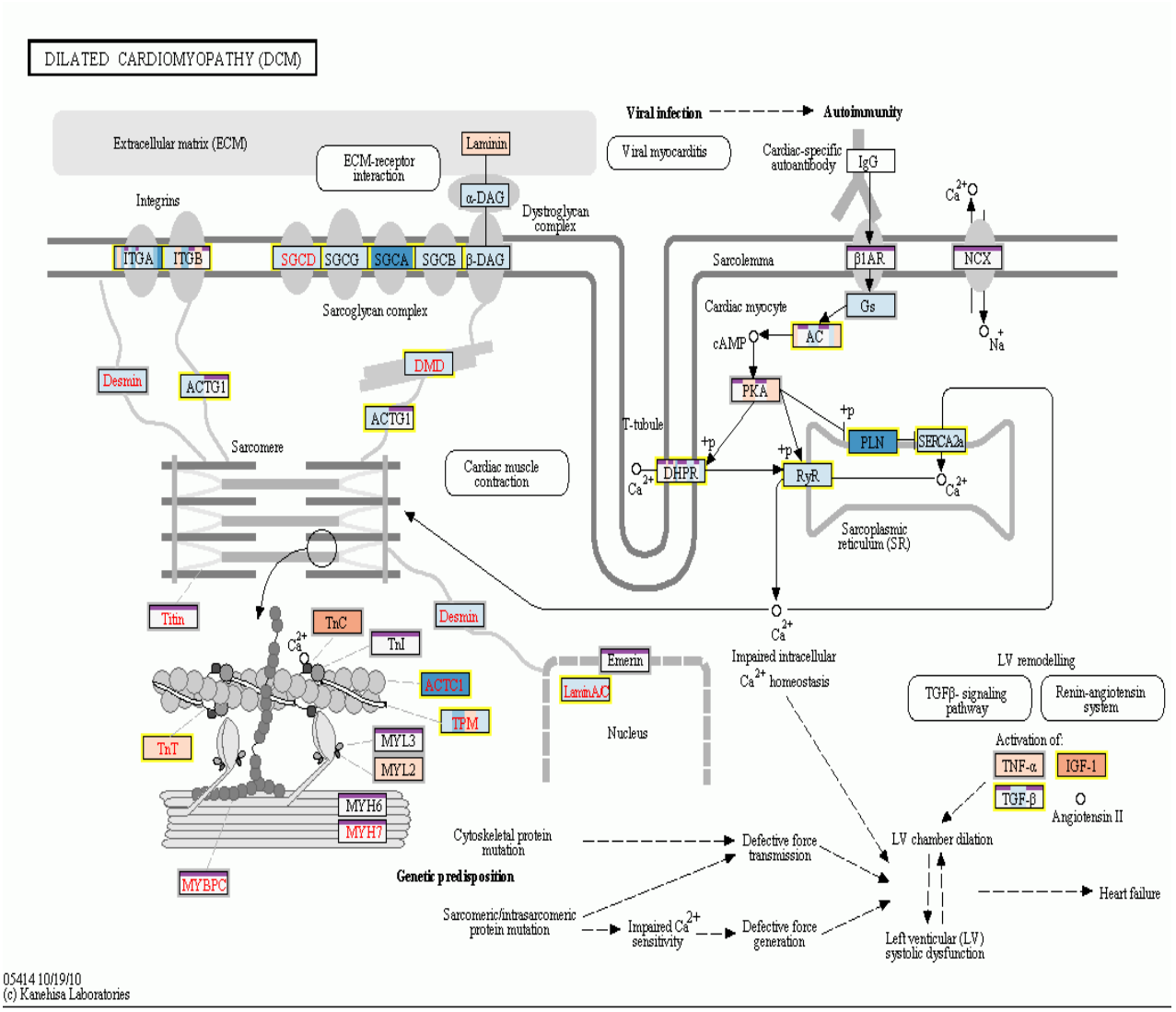
Table 2 Selected pathways and differentially expressed genes

KEGG ID	TITLE	No Genes	No DEG	SPIA p-value	SPIA FDR	SPIA status
Activated pathways						
hsa05414	Dilated cardiomyopathy	91	32	1,46E-11	9,80E-10	Activated
hsa04062	Chemokine signaling pathway	189	45	5,82E-11	2,22E-09	Activated
hsa04060	Cytokine-cytokine receptor interaction	265	55	4,78E-10	1,28E-08	Activated
hsa04270	Vascular smooth muscle contraction	116	34	1,60E-09	3,06E-08	Activated
hsa04670	Leukocyte transendothelial migration	116	32	1,32E-08	2,21E-07	Activated
hsa05166	HTLV-I infection	263	46	2,92E-07	4,34E-06	Activated
hsa04064	NF-kappa B signaling pathway	92	24	2,57E-06	3,13E-05	Activated
hsa05162	Measles	135	27	3,72E-05	0,000416	Activated
hsa04020	Calcium signaling pathway	184	35	6,79E-05	7,00E-04	Activated
hsa05416	Viral myocarditis	71	18	0,000143	0,00137	Activated
hsa04650	Natural killer cell mediated cytotoxicity	138	21	0,000169	0,00151	Activated
hsa05032	Morphine addiction	92	21	0,000196	0,00165	Activated
hsa04380	Osteoclast differentiation	132	26	0,00036	0,00268	Activated
hsa04660	T cell receptor signaling pathway	108	20	0,00108	0,00725	Activated
hsa04330	Notch signaling pathway	47	12	0,00156	0,0095	Activated
Inhibited pathways						
hsa04810	Regulation of actin cytoskeleton	213	48	6,92E-14	9,28E-12	Inhibited
hsa04510	Focal adhesion	201	49	6,62E-11	2,22E-09	Inhibited
hsa05412	Arrhythmogenic right ventricular cardiomyopathy (ARVC	74	27	7,20E-10	1,61E-08	Inhibited
hsa04512	ECM-receptor interaction	84	23	2,25E-06	3,01E-05	Inhibited
hsa05132	Salmonella infection	86	19	0,00029	0,00229	Inhibited
hsa04142	Lysosome	121	24	0,000856	0,00603	Inhibited
hsa05100	Bacterial invasion of epithelial cells	70	16	0,0015	0,0095	Inhibited
hsa04540	Gap junction	89	16	0.00238	0.0122	Inhibited
hsa04070	Phosphatidylinositol signaling system	81	16	0.00961	0.0322	Inhibited

KEGG ID	Kyoto encyclopeadia of genes and genomes pathway ID
Name	KEGG pathway name
No Genes	Number of genes detected in the experiment and belonging to the pathway
No DEG	Number of differentially expressed genes detected in the experiment and belonging to the pathway
SPIA p-value	SPIA global pathway significance p-value
SPIA FDR	FDR corrected SPIA global pathway significance p-value
SPIA status	SPIA estimate on pathway activation/inhibition

Figure 2

Pathway of dilated cardiomyopathy



Legend:

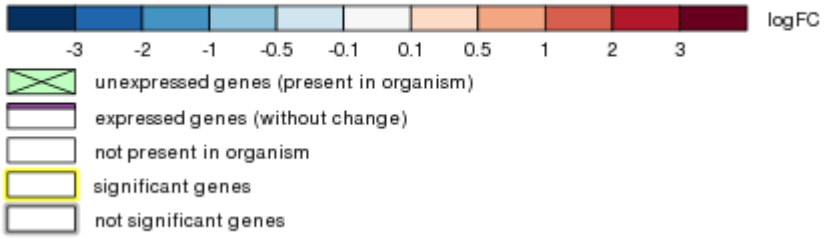
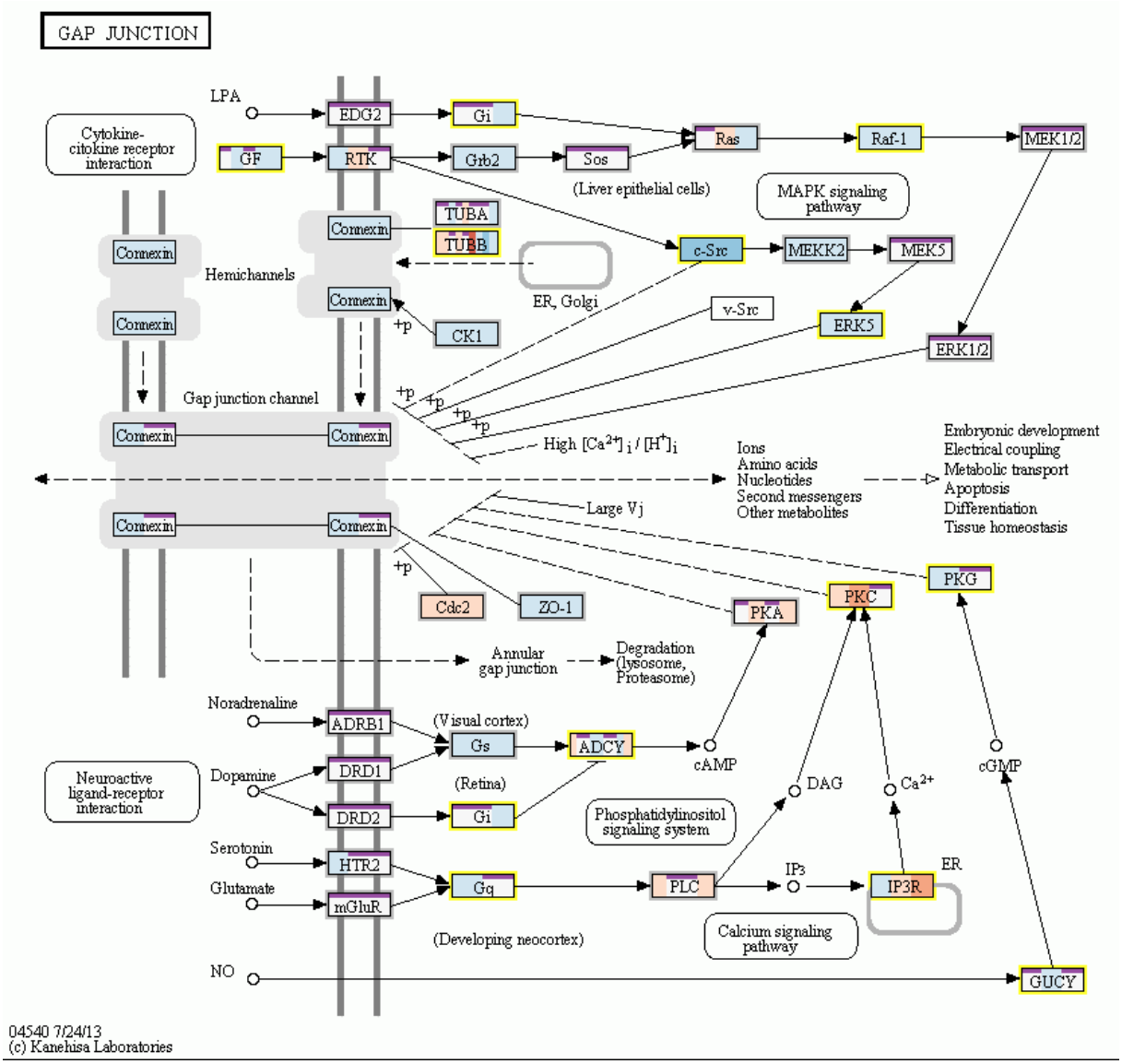


Figure 3

Pathway of Gap junctions



Legend:

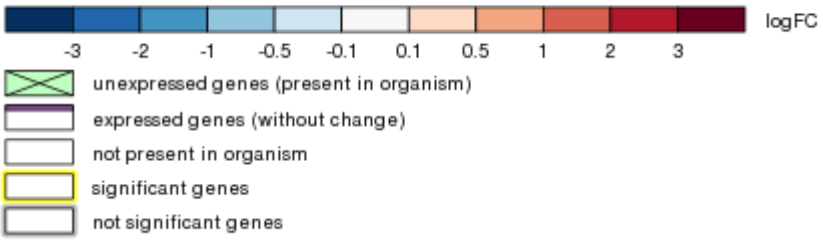
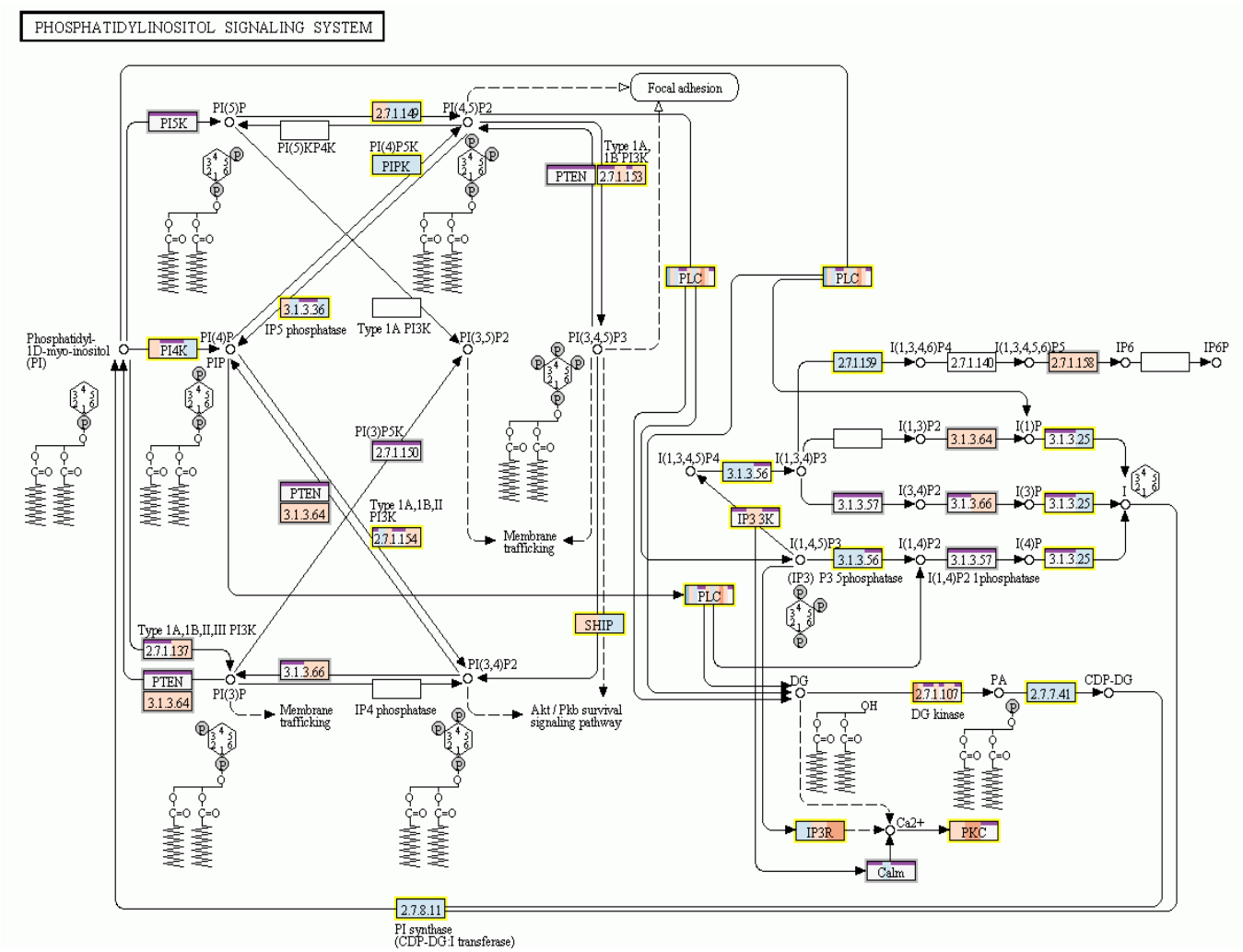


Figure 4

Pathway of phosphatidylinositol signalling system



Legend: