

## Editorial Focus

# Omics meets hypothesis-driven research

## Partnership for innovative discoveries in vascular biology and angiogenesis

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### Summary

The emergence of omics technologies allowing the global analysis of a given biological or molecular system, rather than the study of its individual components, has revolutionized biomedical research, including cardiovascular medicine research in the past decade. These developments raised the prospect that classical, hypothesis-driven, single gene-based approaches may soon become obsolete. The experience accumulated so far, however, indicates that omic technologies only represent tools similar to those classically used by scientists in the past and nowadays, to make hypothesis and

build models, with the main difference that they generate large amounts of unbiased information. Thus, omics and classical hypothesis-driven research are rather complementary approaches with the potential to effectively synergize to boost research in many fields, including cardiovascular medicine. In this article we discuss some general aspects of omics approaches, and review contributions in three areas of vascular biology, thrombosis and haemostasis, atherosclerosis and angiogenesis, in which omics approaches have already been applied (vasculomics).

### Keywords

Atherosclerosis, endothelial cells, gene expression, proteomics, cancer

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## The omics revolution

Biomedical research is largely based on the scientific method, which by definition is hypothesis-driven: information is gathered and used to formulate a well-defined hypothesis explaining a cause-effect relationship between the different variables observed. An experiment is then designed to test this hypothesis. Ideally, the result of this experiment should tell us whether the initial hypothesis was true or false. The art of the scientific method is to choose the appropriate model and to design the best experiment giving a clear and sound response.

The sequencing of the human genome together with the introduction of new technologies allowing rapid, genome-wide, quantitative analysis of gene expression in cells and tissues (i.e. functional genomics or transcriptomics) were perceived as a major revolution in biomedical research, since they opened a whole new spectrum of possibilities in how biological questions may be addressed. Using minute amounts of starting material we

can nowadays monitor the expression of the entire transcriptome in one single experiment, which was not possible a decade ago. Subsequently, other holistic approaches aimed at studying entities in groups or aggregates (“omic”) have been introduced and developed more or less successfully, including epigenomics, proteomics, phenomics, cytomics, ligandomics, metabolomics, epitomics, glycomics, pharmacogenomics, kinomics, physiomics, regulomics, vasculomics and more (1). The launching of a journal (OMICS)<sup>1</sup> dedicated to integrate omics results into biology, further emphasizes the necessity to communicate results in this emerging field of research. The concept beyond omics approaches is that a given biological or molecular system can be best determined and understood by considering it in its globality, rather than studying its components individually (2). This concept is also the founding principle of systems biology in

1 [www.liebertpub.com/publication.aspx?pub\\_id=43](http://www.liebertpub.com/publication.aspx?pub_id=43)

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which a given system is monitored in its entirety in response to changes so that it can be better modeled and thoroughly understood compared to a single pathway approach. Accordingly, many systemic biology studies are based on omics technologies (3). Omics studies generates high-dimensional data sets, which complicates the analysis requiring the massive implication of mathematical, statistical and computational efforts (i.e. bioinformatics) and the development of novel analytical tools (4). For these reasons researchers need to team up with bioinformaticians and biostatisticians in order to fully benefit from their omics experiments. In order to optimize the impact on the success of the experiments, this collaboration should start up-front at the time of the design of the experiment.

## Omics approaches and hypothesis-driven research

From a biomedical perspective, these omics technologies raised the expectation that they may potentially revolutionize our conventional approaches to detect, understand and treat diseases and possibly make the old hypothesis-driven, single gene-based approach obsolete. In other words, scientists traditionally focused on one hypothesis and designed specific experiments to test it, while nowadays omics experiments allow to gather huge amounts of information in a single run, thereby opening the possibility to obtain results without the need for an hypothesis. Does this mean that omics approaches imply the end of hypothesis-driven biomedical research? Certainly not. Omics approaches are emerging as rather complementary tools to hypothesis-driven research. Evidence supporting this notion comes from different observations.

### First, omics-based studies have intrinsic limitations

Results obtained from omics experiments consist of lists of genes or proteins characteristic of a given system in a certain state and are purely descriptive. As such they can provide only a “blurred image” of the system under investigation. When properly processed, these data might be sufficient to extract prominent molecular features, such as gene expression signatures, associated with a given biological, pathological or clinical feature. While this approach promised rapid advances in clinical medicine, impact on everyday clinical practice has been so far marginal (5). Although omics results give us a genome-wide view of the elements present in a system, they tell us little about the way they interact to each other and how they determine the biological characteristics of the analyzed system (i.e. knowing the players only gives us a rough idea of the game they will play). In other words, mere omics results tell us little about mechanisms and it is only through classical hypothesis-driven research that mechanisms can eventually be dissected.

### Second, by integrating multiple datasets omics-generated results can feed hypothesis-driven research

Individual omics experiments produce large amounts of information. Different types of omics approaches (e.g. functional genomics, proteomics, metabolomics) provide complementary information, thereby increasing the complexity of the description

of a biological entity and generating huge databases. With the accumulation of omics data in public repository, such as Gene Expression Omnibus (GEO), it is now becoming possible to extract information from one dataset, encapsulate this information in the form of an expression signature and test its association with a clinical or biological parameter of interest within independent datasets. This new research paradigm for life-science research has proven its value in cancer research. Here is an example to illustrate this concept. A new breast cancer sub-type termed “molecular apocrine” and characterized by high-level of expression of the androgen receptor gene was recently reported (6). Since androgen receptor expressed in prostate cancer is biologically active and promotes cancer progression, this observation raised the question of whether this receptor may also be biologically active in breast cancer and could therefore represent a therapeutic target. To address this question, we used an analytical tool, the Gene Set Enrichment Algorithm (GSEA), based on a computational method that determines whether an *a priori* defined set of genes are differentially expressed between the different conditions tested (7, 8). This approach can effectively be used to assess the functional strength between the expression of a particular gene (in this case the androgen receptor) and its known target genes, i.e. genes whose transcription was shown to be under the influence of this particular gene. The *a priori* defined list of genes was taken from a previous study in which androgen target genes were identified (9). Results of the GSEA experiment showed that the androgen receptor was indeed functionally active in the molecular apocrine tumor group (6). The presence the molecular apocrine subgroup overexpressing the androgen receptor was later confirmed by another group in an independent cohort of patients (10). Furthermore, these authors have used a breast cancer cell line (i.e. MDA-MB453) with a molecular signature characteristic of the apocrine phenotype implanted in mice, to demonstrate that the proliferation *in vivo* of these cells was androgen-dependent. These observations lead to the design of a new phase 2 clinical trial where breast cancer patients expressing biomarkers specific for molecular apocrine are treated with an androgen antagonist. Such an approach may be easily transferred in the future to vasculomics research. This example, illustrates well how omics approaches open up fully novel perspectives, which were hardly imaginable only a decade ago. In this perspective the current situation is reminiscent of the early days of microscopic cellular imaging, whereby the morphological profile of a cell constituted the basic information to formulate a hypothesis linking cell morphology and cell function. Today’s “omic” molecular profiling of the same cell serves the same purpose but at a much higher resolution.

### Third, omics approaches may generate unexpected results that could not have been anticipated by hypothesis-driven research

This, again, is well illustrated in cancer research. For diagnostic, prognostic and therapeutic purposes cancers have been traditionally classified based on histopathological features (TNM classification) (11). The discovery that signaling pathways are deregulated in cancer cells, mostly secondary to genetic events, such as the loss of tumor suppressor genes (e.g. p53 or E-cadherin) or the activation of proto-oncogenes into oncogenes (e.g. Ras

or c-Abl), opened up the possibility of classifying cancers, or at least some of their features, through specific molecular events (12), and leading to the notion of the multistep nature of cancer development and progression (13). This approach, however, depends on the discovery of individual genetic or biochemical events altered in a given tumor. The emergence of functional genomics allowed for the first time an unbiased approach to the molecular classification of cancer, with diagnostic and prognostic implications (14). Moreover, this approach has allowed predicting the progression and response, or the lack thereof, to therapy of some cancers (15). Compared to gene expression profiling, proteomic approaches to cancer diagnosis, prognosis and therapy are still in their infancy. Nevertheless, it is likely that proteomics will become relevant in the near future, for instance by validating at the protein level results obtained in gene expression studies (16, 17).

In short, omics and hypothesis-driven research effectively complement each other: omics techniques produce information, hypothesis-driven research sets the stage, puts order and interprets the information which, on the basis of these new acquisitions, allows formulating new hypotheses to be tested in classical hypothesis-driven experiments. Conversely, omics techniques may be used to test hypotheses that could hardly be tested with a single gene approach. A non-exhaustive list of web pages relevant to omics research is given in Table 1.

## Vasculomics: omics approaches in vascular biology and cardiovascular research

Vascular biology and cardiovascular medicine have already largely exploited omics approaches, which, coupled with classical hypothesis-driven research, have provided new insights into mechanisms of diseases and are generating potential novel diagnostic and prognostic tools. To illustrate this notion, we will briefly highlight the contribution of functional genomics and proteomics to the advancement of thrombosis and haemostasis, atherosclerosis, and angiogenesis research.

### Thrombosis and haemostasis

The haemostatic system, comprising the vasculature, circulating platelets, coagulation proteins, and fibrinolytic mechanisms, is highly complex and tightly regulated (18). The characterization of plasma proteins through standard biochemical methods (e.g. chromatography, 1D and 2D gel electrophoresis, peptide mapping) lead to the identification of many 'factors' mediating pro- and anti-coagulation events. Concomitantly, these studies lead to the discovery that many bleeding and thrombotic disorders were caused by the absence of some of these factors (e.g. factor VIII or VWF in haemophilia A or von Willebrand disease) or to the presence of dysfunctional factors, as it can occur through gene polymorphism (e.g. factor V Leiden, prothrombin G20210A) (19). Since the advent of proteomics, a huge effort has been deployed in the cataloging of proteins from body fluids. Among different body fluids, plasma has been the most studied: from 40 proteins identified in 1977 (20), the HUPO Plasma Proteome Project now holds a core consensus dataset of 3,020 non-redundant identified proteins (21). Out of these proteins, 345 (11%) were annotated as

proteins with cardiovascular-related functions based of published information (22), consistent with the relevance of the plasma proteome to cardiovascular physiology and pathology including the assessment of thrombotic risk (23). Because of their central role in clotting, platelets have also been intensively investigated by classical biochemical methods first and, more recently, by proteomics and also functional genomics approaches (24, 25). However, this flood of data has to date resulted in only marginal gain for clinical practice. The reasons for this 'dead valley' between basic research (or rather the brute force acquisition of proteomic data) and translational medicine lie in the difficulty of extracting valuable biological information from the data. Moreover, the way samples are processed may have a profound influence on the observed profiles of peptides and proteins, a difficulty that has now been relatively well documented (26). Also, a considerable amount of low-molecular-weight proteolytic fragments may appear during sample processing (27). The plethora of possible *in vitro* artifacts makes it very difficult to extract valuable biological information from large-scale body fluid proteomics beyond protein cataloging. Trying to establish standard procedures for sample collection, handling, and preparation can rapidly become an extremely costly and difficult task in itself. Despite these limitations, proteomic approaches have proved useful in characterizing proteins in coagulation sciences (28, 29) and might be particularly efficient in identifying proteins present in both microparticles and secretory microvesicles (30). Microparticles, also known as microvesicles or ectosomes, are small phospholipid vesicles of less than 1  $\mu\text{m}$  in size that are released from a variety of cells such as platelets, red and white blood cells, or endothelial cells, and that contain a subset of proteins derived from their parent cells. For a long time, microparticles have been considered as being cell fragments or "debris" without any biological function. Nowadays they appear to be real cellular effectors involved in a broad spectrum of biological activities such as haemostasis, thrombosis, inflammation, transfer of surface proteins or even angiogenesis (31). Thus, considering the importance of the proteins present in microparticles or in secretory microvesicles, and the role of platelets and endothelial cell secretomes (i.e. the proteins secreted by platelets as well as by vascular cells) in haemostasis, the relevance of proteomics in vascular research will steadily increase in the future (32).

In conclusion, omics should be integrated as an obliged approach to evaluate the interactions among different genes as well as between genes and other acquired factors accounting for the phenotype variability of most coagulation disorders, keeping in mind that most frequently, the clinical skills of the investigators are the major factors allowing identification of the various phenotypes.

### Atherosclerosis

Atherosclerosis, with its subsequent thromboembolic complications is the primary cause of death in Western countries, but their underlying cellular and molecular mechanisms remain largely elusive. The complex nature of atherosclerotic diseases demands the development of novel technologies enabling the discovery of new biomarkers for early detection and the unraveling of underlying molecular mechanisms. In recent years, functional genomics, metabolomics and proteomics have pro-

**Table 1: Non-exhaustive list of useful web pages providing access to annotation and analytical tools and databases frequently used functional genomics and proteomics studies.**

Name	Category	Description	URL
Source	Annotation	Unification tool collecting and compiling data from many scientific databases, to encapsulate the genetics and molecular biology of genes from the genomes of <i>Homo sapiens</i> , <i>Mus musculus</i> , <i>Rattus norvegicus</i> into easy to navigate GeneReports	<a href="http://source.stanford.edu/cgi-bin/source/sourceSearch">http://source.stanford.edu/cgi-bin/source/sourceSearch</a>
The Gene Ontology (GO)	Annotation	Project aimed at providing a controlled vocabulary to describe gene and gene product attributes in any organism	<a href="http://www.geneontology.org/">http://www.geneontology.org/</a>
Netaffx	Annotation	The NetAffx™ Analysis Center at Affymetrix enables researchers to correlate their GeneChip® array results with array design and annotation information	<a href="http://www.affymetrix.com/analysis/index.affx">http://www.affymetrix.com/analysis/index.affx</a>
Uniprot	Annotation	Uniprot provides a comprehensive, high-quality and freely accessible resource of protein sequence and functional information	<a href="http://www.uniprot.org/">http://www.uniprot.org/</a>
Prosite	Annotation	PROSITE consists of documentation entries describing protein domains, families and functional sites as well as associated patterns and profiles to identify them.	<a href="http://expasy.org/prosite/">http://expasy.org/prosite/</a>
Interpro	Annotation	InterPro is a database of protein families, domains, repeats and sites in which identifiable features found in known proteins can be applied to new protein sequences.	<a href="http://www.ebi.ac.uk/interpro/">http://www.ebi.ac.uk/interpro/</a>
Database for Annotation, Visualization and Integrated Discovery (DAVID)	Analysis	DAVID provides a comprehensive set of functional annotation tools to understand biological meaning behind large list of genes	<a href="http://david.abcc.ncifcrf.gov/">http://david.abcc.ncifcrf.gov/</a>
The Eukaryotic Promoter Database	Analysis	Annotated non-redundant collection of eukaryotic POL II promoters, for which the transcription start site has been determined experimentally	<a href="http://www.epd.isb-sib.ch/">http://www.epd.isb-sib.ch/</a>
Cleanex	Analysis	CleanEx is a curated database which provides access to public gene expression data via unique approved gene symbols and which represents heterogeneous expression data produced by different technologies in a way that facilitates joint analysis and cross-dataset comparisons	<a href="http://www.cleanex.isb-sib.ch/">http://www.cleanex.isb-sib.ch/</a>
Eisen cluster	Analysis	Cluster and TreeView are an integrated pair of programs for analyzing and visualizing the results of complex microarray experiments. Both written by Michael Eisen.	<a href="http://rana.lbl.gov/EisenSoftware.htm">http://rana.lbl.gov/EisenSoftware.htm</a>
R	Analysis	R is a free software environment for statistical computing and graphics	<a href="http://www.r-project.org/">http://www.r-project.org/</a>
Bioconductor	Analysis	Bioconductor is an open source and open development software project for the analysis and comprehension of genomic data	<a href="http://www.bioconductor.org/">http://www.bioconductor.org/</a>
Significance Analysis of Microarrays (SAM)	Analysis	Supervised learning software for genomic expression data mining	<a href="http://www.bioconductor.org/">http://www.bioconductor.org/</a>
Biometric research branch arrays tools (BRB)	Analysis	Integrated package for the visualization and statistical analysis of DNA microarray gene expression data	<a href="http://linus.nci.nih.gov/BRB-ArrayTools.html">http://linus.nci.nih.gov/BRB-ArrayTools.html</a>
GSEA (Gene Set Enrichment Algorithm)	Analysis	GSEA is a computational method that determines whether an a priori defined set of genes shows statistically significant, concordant differences between two biological states (e.g. phenotypes)	<a href="http://www.broad.mit.edu/gsea/">http://www.broad.mit.edu/gsea/</a>
Kegg	Analysis	Knowledge-based methods and database for uncovering higher-order systemic behaviors of the cell and the organism from genomic and molecular information.	<a href="http://www.genome.jp/kegg/">http://www.genome.jp/kegg/</a>
Pathway Interaction Database	Analysis	Biomolecular interactions and cellular processes assembled into authoritative human signaling pathways	<a href="http://pid.nci.nih.gov/PID/index.shtml">http://pid.nci.nih.gov/PID/index.shtml</a>
Array express	Database	ArrayExpress is a public repository for transcriptomics data, which is aimed at storing MIAME- and MINSEQE- compliant data in accordance with MGED recommendations	<a href="http://www.ebi.ac.uk/arrayexpress">http://www.ebi.ac.uk/arrayexpress</a>
Stanford MicroArray Database (SMD)	Database	Collection of gene expression data sets in different species	<a href="http://genome-www5.stanford.edu/">http://genome-www5.stanford.edu/</a>
Open Proteomics Database (OPD)	Database	OPD is a public database for storing and disseminating mass spectrometry based proteomics data. The database currently contains roughly 3,000,000 spectra representing experiments from 5 different organisms.	<a href="http://bioinformatics.icmb.utexas.edu/OPD/">http://bioinformatics.icmb.utexas.edu/OPD/</a>
Plasma Proteome Database (PPD)	Database	PPD is a comprehensive, curated, database includes information pertaining to isoform specific expression, disease, localization, post translational modification and single nucleotide polymorphism.	<a href="http://www.plasmaproteome-database.org/">http://www.plasmaproteome-database.org/</a>

vided novel perspectives to these unmatched needs (33). Microarray-based gene expression studies of atherosclerotic lesions, in combination with laser capture microdissection, have been performed to analyze the pathogenic mechanisms associated with atherosclerosis risk factors, including ageing, hypertension, obesity, and viral infection. Most notably, gene expression studies have identified a number of transcription factors differentially expressed in endothelial cells, smooth muscle cells or monocytes / macrophages contributing to atherogenesis, likely involved in activating or repressing atherogenic programs (34). They include nuclear factor-kappaB (NF- $\kappa$ B), peroxisome proliferation-activating receptors (PPARs), the NGFI-B subfamily of orphan receptors (TR3, MINOR and NOT) (35, 36), and Kruppel-like transcription factors (KLF) (37, 38). Gene profiling of atherosclerotic plaques in ApoE<sup>-/-</sup> mice identified many genes differentially expressed during plaque progression (39). Chemokines, such as monocyte chemoattractant protein (MCP)-1 and -5, or macrophage inflammatory protein (MIP)-1 $\alpha$ , -1 $\beta$ , and -2, and matrix degradation enzymes, such as cathepsin-S and matrix metalloproteinase-2, were found associated with disease progression. Remarkably, antibody-mediated inhibition of MCP-1 and -5 in ApoE<sup>-/-</sup> mice reduced plaque area and macrophage content and increased collagen content, resulting in plaque stabilization. In addition, gene expression patterns during the development of in-stent restenosis and in response to statin therapy have been reported (33).

Using proteomics and metabolomics approaches to characterize Sca-1<sup>+</sup> smooth muscle progenitors in ApoE<sup>-/-</sup> mice, it was found that smooth muscle cells derived from Sca-1<sup>+</sup> progenitors had an improved glucose uptake, reduced interleukin (IL)-6 production and upregulated insulin-like growth factor binding proteins (IGFBP) 3 and 6 expression, compared to smooth muscle cells derived from stem cells of wild-type mice. The functional significance of these observations to atherosclerosis was demonstrated through interventional studies in normo- and hypercholesterolemic mice (40).

A biotin protein labeling approach *in vivo*, coupled with avidin-based affinity isolation, SDS-PAGE, and LC-MS/MS analysis, identified 81 proteins mostly involved in immune and inflammatory responses, cell adhesion, and lipid metabolism, whose expression was altered in atherosclerotic tissue (41).

Gene expression studies have also been applied to characterize changes in atherosclerotic lesions in atherosclerosis-prone mice in response to plasma lipid lowering regimes (42). Such studies led to the identification of a network of atherosclerosis genes that in response to lowering plasma cholesterol levels halted plaque progression. These studies illustrate well the power of functional genomics in identifying candidate therapeutic targets that can be subsequently validated in hypothesis-driven experiments.

Since atherosclerotic plaque rupture triggers the acute onset of cardiovascular complications, such as myocardial infarction and stroke, it is important to characterize the biochemical composition of plaques. Proteomics approaches have been recently applied to experimental models and human tissues with the purpose of elucidating the mechanisms of plaque formation and rupture and to identify biomarkers reflecting (or predicting) these events (43). Classical proteomics approaches such as two-

dimensional (2D) gel electrophoresis followed by mass spectrometry (MS) lead to the identification of proteins mediating migration of vascular smooth muscle cells, matrix degradation and inflammation contributing to plaque rupture (44, 45). Through a direct tissue proteomic approach, over 800 proteins present in human coronary atherosclerotic plaques were identified, thereby providing the first large-scale proteomics map of human atherosclerotic plaques (46). Analysis of plasma from patients with peripheral arterial disease by surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF) MS, identified  $\beta$ 2-microglobulin as a biomarker for peripheral arterial disease (47). The characterization of proteins released by atherosclerotic arterial walls in the supernatant of cultured normal and diseased arteries was used as an approach to identify proteins, which may serve as biomarkers of ongoing plaque formation (48).

Proteomics approaches have also been used to map proteins in high- and low-density lipoprotein (HDL and LDL) complexes and to identify novel components with potential pathophysiological relevance (49, 50). Similarly, the protein composition of foam cells has been analyzed to identify potential biomarkers for atherosclerosis (51, 52). Proteomics analyses have been recently associated with metabolomics studies linking alterations of cellular proteins, signal transduction, cellular metabolism and phenotype to more comprehensively address pathophysiological mechanisms of atherosclerosis (53).

In short, vasculomics have already contributed to improve our understanding of the mechanisms involved in the predisposition and pathophysiology of atherosclerosis. It can be anticipated that vasculomics will contribute in the future to the discovery of new genes and pathways representing novel biomarkers or candidate therapeutic targets in atherosclerosis, opening novel opportunities to translate experimental knowledge into clinical practice (54).

### Angiogenesis and vascular remodelling

Over the past decade, angiogenesis has emerged as one of the fast-growing fields in biomedical research. Angiogenesis plays a key role in many physiological and pathological situations, including development, tissue remodeling, wound healing, reproduction, inflammation, cancer and metabolic diseases (e.g. obesity, diabetes) (55). In recent years many cellular and molecular mechanisms of angiogenesis have been unraveled, and transcriptomics and proteomic approaches contributed to this progress (56). Functional genomics and proteomics have been combined with forward and reverse genetic screens to identify candidate therapeutic targets for pro- and anti-angiogenic strategies (57). Gene expression profiling studies were used to characterize the genetic programs activated by different angiogenic factors, such as VEGF, PlGF or FGF2. Remarkably, different factors were found to induce expression of distinct sets of genes with only little overlap (58–60). A number of studies used transcriptomics approaches to systematically compare gene expression in endothelial cells isolated from normal, tumor and regenerating blood vessels. Collectively, these studies revealed that depending on their origin and activation state, endothelial cells initiate different gene expression programs, thereby revealing differences between physiological and pathological angiogenesis potentially important for the development of tumor-specific, vascular-tar-

geted therapies or for diagnostic and monitoring purposes (61, 62, 63, 64).

In an elegant study, Abdollahi et al. used a genome-wide expression profiling and phosphorylation analysis approach to demonstrate that in human microvascular endothelium, endostatin, an endogenous inhibitor of angiogenesis, down-regulates angiogenic signaling pathways and up-regulates the expression of anti-angiogenic genes (65). This study also identified genes not previously associated with angiogenesis. Gene expression profiling has been used to characterize the molecular features of endothelial cells in specific vascular beds, most notably of the lung (66). Besides classical endothelial cell genes, such as VEGF receptor (VEGFR)-1, VEGFR-2, angiopoietin-2, Tie1 and Tie2, or VE-cadherin, genes involved in vascular development, including (e.g. delta/notch and Wnt), as well as of genes that had little or no previous association with endothelial cells were identified. Gene expression profiling in normal versus tumoral endothelial cells has also been used to identify potential biomarkers for tumor angiogenesis (63, 67, 68).

Proteomics approaches are less advanced in the study of angiogenesis compared to functional genomics, mostly because of current methodological limitations. Nevertheless, they have already delivered interesting results. Proteomics approaches *in vitro* have been used to identify proteins expressed in cultured endothelial cells under normal conditions (69), in response to tumor cells (70), or to pro-apoptotic drugs (71) and to identify novel drug target. For example, by using an affinity capture approach on surface-immobilized tyrosine kinase inhibitor (i.e. SU6668), in combination with MS, the aurora kinases and TANK-binding kinase 1 were identified as unanticipated SU6668 targets (72). This approach demonstrates the potential of chemical proteomics to characterize target specificity of kinase inhibitors. Proteomics approaches were also used to characterize the protein expression profiles at different stages of angiogenesis *in vivo* (73) or in tumor vessels. *In vivo* or *in vitro* biotinylation of luminal-accessible or cell surface proteins, followed by selective enrichment with avidin affinity chromatography coupled with LC-MS-based analysis, was used to identify proteins preferentially expressed in kidney cancer vessels versus normal kidney vessels, and in blood versus lymphatic endothelial cells (74, 75). In another approach, endothelial cells were isolated from human non-small cell lung cancers and normal lung tissue by immunomagnetic beads. Proteins were analyzed by one-dimensional gel electrophoresis, tryptic digestion and LC-electrospray ionization (ESI) ion trap tandem MS. Of the hundreds of proteins identified in each sample, 16 were present in the majority of samples and retained for further analysis. Peroxiredoxin 4, thymopoietin, coatomer and protein complex  $\gamma$  subunit were subsequently validated in a larger patient set (76). In an elegant study, isotope-coded affinity tag-labeled proteins extracted from tissues of angiogenesis-impaired MMP-2<sup>-/-</sup> mice and angiogenesis-competent wt mice, were analyzed by 2D-LC and tandem MS to identify novel MMP-2 targets (77). Insulin-like growth factor binding protein 6, follistatin-like 1, cystatin C, pleiotrophin and connective tissue growth factor (CTGF) were identified as novel MMP-2 substrates. Importantly, cleavage of CTGF and pleiotrophin by MMP-2 released bound and inactivated VEGF.

Combination of state-of-the-art proteomics technologies and *in vivo* imaging is emerging as a new integrative approach to accelerate validation of newly discovered vascular targets for diagnostic and therapeutic purposes (78).

Functional genomics and proteomics approaches were also applied to identify vascular wall proteins as mediators or markers of vascular remodeling. Nogo-B was identified through a classical proteomics approach as a protein enriched in caveolae/lipid rafts of vascular endothelial and smooth muscle cells. Functional experiments have revealed that mice lacking Nogo-B have exaggerated neointimal proliferation upon endothelial injury, thereby implicating Nogo-B as regulator of vascular remodeling (79). SELDI-TOF MS was used to study changes in proteins released by the aorta of rat strains with different susceptibilities to hypertension and receiving a hypertensive drug (L-NAME). Ubiquitin, smooth muscle (SM) 22alpha, thymosin  $\beta$ 4, and C-terminal fragment of filamin A, were found to be differentially secreted in hypertensive-prone rats in response to L-NAME and were shown to correlate with aortic wall hypertrophic remodeling (80).

In short, functional genomics approaches have generated large amounts of novel and in part unexpected data on the molecular characteristics of endothelial cells and tumor angiogenesis that serve as a basis to formulate novel hypotheses, address mechanisms and identify candidate therapeutic targets or biomarkers.

## Simple animal models for vasculomic research

Much of experimental research in vascular development, angiogenesis and remodeling, including omics-based research, has been conducted in mammals, in particular mice and rats. These complex animal models have valuable features (e.g. possibility of genetic manipulations or therapeutic interventions), making them interesting models for addressing topics of clinical relevance such as tumor angiogenesis, atherosclerosis, or arterio-genesis. Because of their anatomical and functional complexity, these animal models, however, have limitations for other applications, such as genome-wide target identification, rapid functional target validation, or high-throughput drug screens. For this reason a number of simple animal models have emerged in vascular and angiogenesis research in recent years, most notably the chicken embryo (i.e. chorioallantoic membrane – CAM), *Danio rerio* (zebrafish), and *Xenopus laevis* (African clawed frog) tadpoles.

Thanks to the possibility to directly monitor vessel formation over time and to easily access it (to deliver drugs or to recover tissue), the CAM model has been used for many years (81). Recently, genomic approaches (i.e. microarray-based screens) have been applied in this model to dissect mechanisms of tumor angiogenesis (82), and proteomics-based strategies are underway.

Zebrafish has been successfully used in mutagenesis-based genetic screens (83), morpholino oligonucleotide-mediated knockdowns experiments (84), drug screens and target-validation experiments (85, 86), to identify new mediators of angiogenesis or to dissect specific signaling pathways, including Hedgehog, Notch, wnt, VEGFs/VEGFRs, FGF (87, 88). A significant feature of this model is the possibility of performing real-

time *in vivo* imaging of vessels in the whole embryo, using transgenic fish expressing GFP in the vessels, which greatly facilitates morphological and phenotypical screens (89). Proteomics approaches have been applied in zebrafish to study embryonic development (90), to identify phosphoproteins (91) and to characterize the effect of hypoxia in muscles (92), and it is anticipated that applications in vascular development will follow soon. Zebrafish were recently used to investigate tumor angiogenesis by grafting mammalian tumor cells (93, 94), thereby opening new possibilities to omics research in tumor angiogenesis.

*Xenopus laevis* tadpoles are being increasingly used as a further non-mammalian model for the identification and validation of molecular targets and mechanisms of angiogenesis and lymphangiogenesis. In general, interventions consist in the modulation of the expression of proteins of interest (by the delivery of cDNA, RNAi, or antibodies), or drug testing (87, 88). Omics technologies have been applied to *Xenopus laevis*, but only rarely to the study of angiogenesis. For example, a DNA microarray screening of vascular endothelial cells derived from *Xenopus* animal cap and treated with activin and angiopoietin-2, lead to the identification of XRASGRP2, a gene encoding an homologue of human RASGRP2, which is required for haematopoietic and vascular development (95). Similarly, proteomics technologies have been applied to *Xenopus laevis* (including eggs) but not yet to study vascular development or angiogenesis (96).

In short, chicken CAM, zebrafish and *Xenopus laevis* models have already greatly contributed to the study of vascular development and angiogenesis. Omics technologies have been applied to them, but significantly less compared to mouse and human models and tissues. Considering several intrinsic features of these simple models, it can be anticipated that omics approaches will be more widely applied to them in the future.

## Outlook

Omic technologies, in particular functional genomics and proteomics, have profoundly influenced our ability to characterize

and monitor complex biological events with wide-reaching implications on the way biomedical research, including cardiovascular medicine and angiogenesis research, is carried out today. The ability to monitor thousands of genes and hundreds of proteins in one single experiment may be mistakenly taken as a suggestion to perform experiments without the need for a clear hypothesis! One may expect that among the many data generated by omic experiments, some will be eventually useful! While this attitude may be tempting, it is certainly not the right one: omic approaches cannot be reduced to "fishing" experiences in unfamiliar seas. In fact omic technologies represent tools similar to those that scientists classically used in the past and still use today, to make hypotheses and build models, with the difference that they generate large amounts of unbiased information allowing to distinguish more details of the big picture. However, if these tools are not properly directed by a well-defined hypothesis it is unlikely that the information they provide will generate useful results. Thus, for omics and classical hypothesis-driven research to effectively synergize and boost research advancement in cardiovascular medicine and angiogenesis research, studies should be performed in carefully chosen systems and controlled conditions that have a good chance to answer the specific questions asked. Obtained data should be submitted to a severe bioinformatic analysis to extract significant information. Creative data processing combined with a skilled and sensitive researcher's eye, may disclose unexpected results, which may be used to formulate new hypotheses, thus expanding the exploration field. In this perspective omics approaches are emerging as inspiring companions to hypothesis-driven research in vascular biology and many other specialties.

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## References

- Weinstein JN 'Omic' and hypothesis-driven research in the molecular pharmacology of cancer. *Curr Opin Pharmacol* 2002; 2: 361–365.
- Evans GA Designer science and the „omic“ revolution. *Nat Biotechnol* 2000; 18: 127.
- Ideker T, Galitski T, Hood LA A new approach to decoding life: systems biology. *Annu Rev Genomics Hum Genet* 2001; 2: 343–372.
- Yu U, Lee SH, Kim YJ, et al. Bioinformatics in the post-genome era. *J Biochem Mol Biol* 2004; 37: 75–82.
- Sotiriou C, Piccart MJ Taking gene-expression profiling to the clinic: when will molecular signatures become relevant to patient care? *Nat Rev Cancer* 2007; 7: 545–553.
- Farmer P, Bonnefoi H, Becette V, et al. Identification of molecular apocrine breast tumours by microarray analysis. *Oncogene* 2005; 24: 4660–4671.
- Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci USA* 2005; 102: 15545–15550.
- Mootha VK, Lindgren CM, Eriksson KF, et al. PGC-1 $\alpha$ -responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat Genet* 2003; 34: 267–273.
- Nelson PS, Clegg N, Arnold H, et al. The program of androgen-responsive genes in neoplastic prostate epithelium. *Proc Natl Acad Sci USA* 2002; 99: 11890–11895.
- Doane AS, Danso M, Lal P, et al. An estrogen receptor-negative breast cancer subset characterized by a hormonally regulated transcriptional program and response to androgen. *Oncogene* 2006; 25: 3994–4008.
- Gospodarowicz M, Benedet L, Hutter RV, et al. History and international developments in cancer staging. *Cancer Prev Control* 1998; 2: 262–268.
- Hanahan D, Weinberg RA The hallmarks of cancer. *Cell* 2000; 100: 57–70.
- Vogelstein B, Kinzler KW The multistep nature of cancer. *Trends Genet* 1993; 9: 138–141.
- Liu ET Classification of cancers by expression profiling. *Curr Opin Genet Dev* 2003; 13: 97–103.
- Nuyten DS, van de Vijver MJ Using microarray analysis as a prognostic and predictive tool in oncology: focus on breast cancer and normal tissue toxicity. *Semin Radiat Oncol* 2008; 18: 105–114.
- Alaiya AA, Franzen B, Auer G, et al. Cancer proteomics: from identification of novel markers to creation of artificial learning models for tumor classification. *Electrophoresis* 2000; 21: 1210–1217.
- Ludwig JA, Weinstein JN Biomarkers in cancer staging, prognosis and treatment selection. *Nat Rev Cancer* 2005; 5: 845–856.
- Troy GC An overview of hemostasis. *Vet Clin North Am Small Anim Pract* 1988; 18: 5–20.
- Ferrer-Antunes C Polymorphisms of coagulation factor genes—a review. *Clin Chem Lab Med* 1998; 36: 897–906.
- Anderson L, Anderson NG High resolution two-dimensional electrophoresis of human plasma proteins. *Proc Natl Acad Sci USA* 1977; 74: 5421–5425.
- Omenn GS. The Human Proteome Organization Plasma Proteome Project pilot phase: reference speci-

- mens, technology platform comparisons, and standardized data submissions and analyses. *Proteomics* 2004; 4: 1235–1240.
22. Berhane BT, Zong C, Liem DA, et al. Cardiovascular-related proteins identified in human plasma by the HUPO Plasma Proteome Project pilot phase. *Proteomics* 2005; 5: 3520–3530.
23. Scully MF Plasma peptidome: A new approach for assessing thrombotic risk? *Thromb Haemost* 2006; 96: 697.
24. Dittrich M, Birschmann I, Stuhlfelder C, et al. Understanding platelets. Lessons from proteomics, genomics and promises from network analysis. *Thromb Haemost* 2005; 94: 916–925.
25. Dittrich M, Birschmann I, Pfrang J, et al. Analysis of SAGE data in human platelets: features of the transcriptome in an anucleate cell. *Thromb Haemost* 2006; 95: 643–651.
26. Barelli S, Cretaz D, Thadikkaran L, et al. Plasma/serum proteomics: pre-analytical issues. *Expert Rev Proteomics* 2007; 4: 363–370.
27. Ayache S, Panelli M, Marincola FM, et al. Effects of storage time and exogenous protease inhibitors on plasma protein levels. *Am J Clin Pathol* 2006; 126: 174–184.
28. Tammen H, Mohring T, Kellmann M, et al. Mass spectrometric phenotyping of Val34Leu polymorphism of blood coagulation factor XIII by differential peptide display. *Clin Chem* 2004; 50: 545–551.
29. Mann KG, Brummel-Ziedins K, Undas A, et al. Does the genotype predict the phenotype? Evaluations of the hemostatic proteome. *J Thromb Haemost* 2004; 2: 1727–1734.
30. Pula G, Perera S, Prokopi M, et al. Proteomic analysis of secretory proteoforms and vesicles in vascular research. *Proteomics Clin Appl* 2008; 2: 882–889.
31. Piccin A, Murphy WG, Smith OP Circulating microparticles: pathophysiology and clinical implications. *Blood Rev* 2007; 21: 157–171.
32. Smalley DM, Root KE, Cho H, et al. Proteomic discovery of 21 proteins expressed in human plasma-derived but not platelet-derived microparticles. *Thromb Haemost* 2007; 97: 67–80.
33. Tuomisto TT, Binder BR, Yla-Herttuala S Genetics, genomics and proteomics in atherosclerosis research. *Ann Med* 2005; 37: 323–332.
34. Monajemi H, Arkenbout EK, Pannekoek H Gene expression in atherogenesis. *Thromb Haemost* 2001; 86: 404–412.
35. Martinez-Gonzalez J, Rius J, Castello A, et al. Neuron-derived orphan receptor-1 (NOR-1) modulates vascular smooth muscle cell proliferation. *Circ Res* 2003; 92: 96–103.
36. Arkenbout EK, van Bragt M, Eldering E, et al. TR3 orphan receptor is expressed in vascular endothelial cells and mediates cell cycle arrest. *Arterioscler Thromb Vasc Biol* 2003; 23: 1535–1540.
37. van Thienen JV, Fledderus JO, Dekker RJ, et al. Shear stress sustains atheroprotective endothelial KLF2 expression more potently than statins through mRNA stabilization. *Cardiovasc Res* 2006; 72: 231–240.
38. Yan FF, Liu YF, Liu Y, et al. KLF4: a novel target for the treatment of atherosclerosis. *Med Hypotheses* 2008; 70: 845–847.
39. Lutgens E, Faber B, Schapira K, et al. Gene profiling in atherosclerosis reveals a key role for small inducible cytokines: validation using a novel monocyte chemoattractant protein monoclonal antibody. *Circulation* 2005; 111: 3443–3452.
40. Mayr M, Zampetaki A, Sidibe A, et al. Proteomic and metabolomic analysis of smooth muscle cells derived from the arterial media and adventitial progenitors of apolipoprotein E-deficient mice. *Circ Res* 2008; 102: 1046–1056.
41. Wu J, Liu W, Sousa E, et al. Proteomic identification of endothelial proteins isolated in situ from atherosclerotic aorta via systemic perfusion. *J Proteome Res* 2007; 6: 4728–4736.
42. Skogsberg J, Lundstrom J, Kovacs A, et al. Transcriptional profiling uncovers a network of cholesterol-responsive atherosclerosis target genes. *PLoS Genet* 2008; 4: e1000036.
43. Blanco-Colio LM, Martin-Ventura JL, Vivanco F, et al. Biology of atherosclerotic plaques: what we are learning from proteomic analysis. *Cardiovasc Res* 2006; 72: 18–29.
44. Sung HJ, Ryang YS, Jang SW, et al. Proteomic analysis of differential protein expression in atherosclerosis. *Biomarkers* 2006; 11: 279–290.
45. Almofti MR, Huang Z, Yang P, et al. Proteomic analysis of rat aorta during atherosclerosis induced by high cholesterol diet and injection of vitamin D3. *Clin Exp Pharmacol Physiol* 2006; 33: 305–309.
46. Bagnato C, Thumar J, Mayya V, et al. Proteomics analysis of human coronary atherosclerotic plaque: a feasibility study of direct tissue proteomics by liquid chromatography and tandem mass spectrometry. *Mol Cell Proteomics* 2007; 6: 1088–1102.
47. Wilson AM, Kimura E, Harada RK, et al. Beta2-microglobulin as a biomarker in peripheral arterial disease: proteomic profiling and clinical studies. *Circulation* 2007; 116: 1396–1403.
48. Duran MC, Martin-Ventura JL, Mas S, et al. Characterization of the human atheroma plaque secretome by proteomic analysis. *Methods Mol Biol* 2007; 357: 141–150.
49. Karlsson H, Leanderson P, Tagesson C, et al. Lipoproteomics II: mapping of proteins in high-density lipoprotein using two-dimensional gel electrophoresis and mass spectrometry. *Proteomics* 2005; 5: 1431–1445.
50. Karlsson H, Leanderson P, Tagesson C, et al. Lipoproteomics I: mapping of proteins in low-density lipoprotein using two-dimensional gel electrophoresis and mass spectrometry. *Proteomics* 2005; 5: 551–565.
51. Yang PY, Rui YC, Yang PY, et al. Proteomic analysis of foam cells. *Methods Mol Biol* 2007; 357: 297–305.
52. Fach EM, Garulacan LA, Gao J, et al. In vitro biomarker discovery for atherosclerosis by proteomics. *Mol Cell Proteomics* 2004; 3: 1200–1210.
53. Mayr M, Madhu B, Xu Q Proteomics and metabolomics combined in cardiovascular research. *Trends Cardiovasc Med* 2007; 17: 43–48.
54. Miller DT, Ridker PM, Libby P, et al. Atherosclerosis: the path from genomics to therapeutics. *J Am Coll Cardiol* 2007; 49: 1589–1599.
55. Carmeliet P Angiogenesis in health and disease. *Nat Med* 2003; 9: 653–660.
56. Mittal V, Nolan DJ Genomics and proteomics approaches in understanding tumor angiogenesis. *Expert Rev Mol Diagn* 2007; 7: 133–147.
57. Korherr C, Gille H, Schafer R, et al. Identification of proangiogenic genes and pathways by high-throughput functional genomics: TBK1 and the IRF3 pathway. *Proc Natl Acad Sci USA* 2006; 103: 4240–4245.
58. Jih YJ, Lien WH, Tsai WC, et al. Distinct regulation of genes by bFGF and VEGF-A in endothelial cells. *Angiogenesis* 2001; 4: 313–321.
59. Abe M, Sato Y cDNA microarray analysis of the gene expression profile of VEGF-activated human umbilical vein endothelial cells. *Angiogenesis* 2001; 4: 289–298.
60. Schoenfeld J, Lessan K, Johnson NA, et al. Bioinformatic analysis of primary endothelial cell gene array data illustrated by the analysis of transcriptome changes in endothelial cells exposed to VEGF-A and PlGF. *Angiogenesis* 2004; 7: 143–156.
61. St Croix B, Rago C, Velculescu V, et al. Genes expressed in human tumor endothelium. *Science* 2000; 289: 1197–1202.
62. Seaman S, Stevens J, Yang MY, et al. Genes that distinguish physiological and pathological angiogenesis. *Cancer Cell* 2007; 11: 539–554.
63. Ghilardi C, Chiorino G, Dossi R, et al. Identification of novel vascular markers through gene expression profiling of tumor-derived endothelium. *BMC Genomics* 2008; 9: 201.
64. Bhati R, Patterson C, Livasy CA, et al. Molecular characterization of human breast tumor vascular cells. *Am J Pathol* 2008; 172: 1381–1390.
65. Abdollahi A, Hahnfeldt P, Maercker C, et al. Endostatin's antiangiogenic signaling network. *Mol Cell* 2004; 13: 649–663.
66. Favre CJ, Mancuso M, Maas K, et al. Expression of genes involved in vascular development and angiogenesis in endothelial cells of adult lung. *Am J Physiol Heart Circ Physiol* 2003; 285: H1917–1938.
67. Hardwick JS, Yang Y, Zhang C, et al. Identification of biomarkers for tumor endothelial cell proliferation through gene expression profiling. *Mol Cancer Ther* 2005; 4: 413–425.
68. Sessa C, Guibal A, Del Conte G, et al. Biomarkers of angiogenesis for the development of antiangiogenic therapies in oncology: tools or decorations? *Nat Clin Pract Oncol* 2008; 5: in press.
69. Bruneel A, Labas V, Mailloux A, et al. Proteomic study of human umbilical vein endothelial cells in culture. *Proteomics* 2003; 3: 714–723.
70. Li A, Li H, Jin G, et al. A proteomic study on cell cycle progression of endothelium exposed to tumor conditioned medium and the possible role of cyclin D1/E. *Clin Hemorheol Microcirc* 2003; 29: 383–390.
71. Bruneel A, Labas V, Mailloux A, et al. Proteomics of human umbilical vein endothelial cells applied to etoposide-induced apoptosis. *Proteomics* 2005; 5: 3876–3884.
72. Godl K, Gruss OJ, Eickhoff J, et al. Proteomic characterization of the angiogenesis inhibitor SU6668 reveals multiple impacts on cellular kinase signaling. *Cancer Res* 2005; 65: 6919–6926.
73. Thompson LJ, Wang F, Proia AD, et al. Proteome analysis of the rat cornea during angiogenesis. *Proteomics* 2003; 3: 2258–2266.
74. Roesli C, Mumprecht V, Neri D, et al. Identification of the surface-accessible, lineage-specific vascular proteome by two-dimensional peptide mapping. *Faseb J* 2008; in press.
75. Castronovo V, Waltregny D, Kischel P, et al. A chemical proteomics approach for the identification of accessible antigens expressed in human kidney cancer. *Mol Cell Proteomics* 2006; 5: 2083–2091.
76. Park HJ, Kim BG, Lee SJ, et al. Proteomic profiling of endothelial cells in human lung cancer. *J Proteome Res* 2008; 7: 1138–1150.
77. Dean RA, Butler GS, Hamma-Kourbali Y, et al. Identification of candidate angiogenic inhibitors processed by matrix metalloproteinase 2 (MMP-2) in cell-based proteomic screens: disruption of vascular endothelial growth factor (VEGF)/heparin affinity regulatory peptide (pleiotrophin) and VEGF/Connective tissue growth factor angiogenic inhibitory complexes by MMP-2 proteolysis. *Mol Cell Biol* 2007; 27: 8454–8465.
78. Simonson AB, Schnitzer JE Vascular proteomic mapping in vivo. *J Thromb Haemost* 2007; 5 (Suppl 1): 183–187.
79. Acevedo L, Yu J, Erdjument-Bromage H, et al. A new role for Nogo as a regulator of vascular remodeling. *Nat Med* 2004; 10: 382–388.
80. Delbosch S, Haloui M, Louedec L, et al. Proteomic analysis permits the identification of new biomarkers

of arterial wall remodeling in hypertension. *Mol Med* 2008; 14: 383–394.

**81.** Ribatti D, Vacca A, Roncali L, et al. The chick embryo chorioallantoic membrane as a model for in vivo research on angiogenesis. *Int J Dev Biol* 1996; 40: 1189–1197.

**82.** Hagedorn M, Javerzat S, Gilges D, et al. Accessing key steps of human tumor progression in vivo by using an avian embryo model. *Proc Natl Acad Sci USA* 2005; 102: 1643–1648.

**83.** Jin SW, Herzog W, Santoro MM, et al. A transgene-assisted genetic screen identifies essential regulators of vascular development in vertebrate embryos. *Dev Biol* 2007; 307: 29–42.

**84.** Lee P, Goishi K, Davidson AJ, et al. Neuropilin-1 is required for vascular development and is a mediator of VEGF-dependent angiogenesis in zebrafish. *Proc Natl Acad Sci USA* 2002; 99: 10470–10475.

**85.** Kidd KR, Weinstein BM Fishing for novel angiogenic therapies. *Br J Pharmacol* 2003; 140: 585–594.

**86.** Rubinstein AL Zebrafish: from disease modeling to drug discovery. *Curr Opin Drug Discov Devel* 2003; 6: 218–223.

**87.** Ny A, Autiero M, Carmeliet P Zebrafish and Xenopus tadpoles: small animal models to study angiogenesis and lymphangiogenesis. *Exp Cell Res* 2006; 312: 684–693.

**88.** De Smet F, Carmeliet P, Autiero M Fishing and frogging for anti-angiogenic drugs. *Nat Chem Biol* 2006; 2: 228–229.

**89.** Lawson ND, Weinstein BM In vivo imaging of embryonic vascular development using transgenic zebrafish. *Dev Biol* 2002; 248: 307–318.

**90.** Link V, Shevchenko A, Heisenberg CP Proteomics of early zebrafish embryos. *BMC Dev Biol* 2006; 6: 1.

**91.** Lemeer S, Pinkse MW, Mohammed S, et al. Online automated in vivo zebrafish phosphoproteomics: from large-scale analysis down to a single embryo. *J Proteome Res* 2008; 7: 1555–1564.

**92.** Bosworth CA, Chou CW, Cole RB, et al. Protein expression patterns in zebrafish skeletal muscle: initial characterization and the effects of hypoxic exposure. *Proteomics* 2005; 5: 1362–1371.

**93.** Nicoli S, Ribatti D, Cotelli F, et al. Mammalian tumor xenografts induce neovascularization in zebrafish embryos. *Cancer Res* 2007; 67: 2927–2931.

**94.** Stoletov K, Klemke R Catch of the day: zebrafish as a human cancer model. *Oncogene* 2008; 27: 4509–4520.

**95.** Nagamine K, Matsuda A, Asashima M, et al. XKR2 expression during early development of Xenopus embryos. *Biochem Biophys Res Commun* 2008; 372: 886–891.

**96.** Sato K, Iwasaki T, Sakakibara K, et al. Towards the molecular dissection of fertilization signaling: Our functional genomic/proteomic strategies. *Proteomics* 2002; 2: 1079–1089.