Editorial

Diabetes and cardiovascular disease: let's push forward with translational research

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Abstract: Albeit advances in therapy have reduced morbidity and mortality in patients with diabetes, cardiovascular (CV) risk is far to be eradicated. This is partially due to the fact that breakthrough therapies have yet to be approved to counteract the atherosclerotic burden in this setting. Therefore, it is very important to understand the molecular mechanisms underpinning diabetes-related CV complications. Growing evidence is supporting the concept that translational research is perhaps the best approach to unveil novel insights into disease etiology and its link with CV phenotypes. The recent employment of high throughput "omics" (i.e., metabolomics, transcriptomics, proteomics) is a clinically relevant approach which may provide insightful interpretations of diabetes-related biological signals. The possibility to analyse thousands or more molecules simultaneously has given "omics" the ability to generate enormous quantities of data which may somehow offer a precious "window on the disease". In the present article, we critically discuss the importance of translational research in diabetes, including potential difficulties which may arise in the implementation and development of promising technologies from the laboratory to the marketplace.

Keywords: Diabetes; cardiovascular (CV) disease; omics; translational research; therapeutics

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Atherosclerotic burden in diabetic people

Prevalence of diabetes mellitus (DM) is increasing rapidly, and individuals with DM are at high risk for cardiovascular (CV) disorders that affect the heart, brain and peripheral vessels (1). The International Diabetes Federation currently estimates that 382 million people are affected by type 2 diabetes (T2D), with a global age-adjusted prevalence of 10%. If these trends continue, 592 million people, or 1 adult in 10, will have diabetes by 2035 (2). Most importantly, a substantial proportion of affected people are unaware of their condition and do not receive treatment. Hence, advances in the diagnosis of DM are of paramount importance to reduce morbidity and mortality in this population. DM is associated with accelerated atherosclerosis occurring at different arterial districts including carotid arteries, aorta, femoral arteries, and lower extremities (3). A recent cohort study including almost 2 million individuals, showed that peripheral artery disease (PAD) is the most common complication observed among patients with T2D with a first CV presentation, being reported in 992 (16, 2%) out of 6,137 patients (4). Of note, PAD in T2D patients was the most prevalent disorder as compared to heart failure, stroke and stable angina, and showed the strongest association with T2D (adjusted HR =2.98, 95% CI, 2.76-3.22). These data strengthen the notion that T2D is significantly associated with atherosclerotic burden and vascular complications.

From mechanisms to treatment

Over the last 20 years, seminal work has significantly contributed to delineate key pathways responsible for endothelial dysfunction, inflammation, and accelerated atherogenesis in experimental models of hyperglycemia and T2D (5). One of the most significant discoveries was that accumulation of free radicals, namely superoxide

anion, is capable to activate an array of cellular pathways including polyol and hexosamine flux, advanced glycation end products (AGEs), protein kinase C (PKC), and NF-κB-mediated vascular inflammation (6). Of note, normalization of mitochondrial reactive oxygen species (ROS) by inhibiting electron transport chain complexes prevented glucose-induced activation of PKC, AGEs formation, sorbitol accumulation and NF-κB-dependent transcriptional programs (6). Of interest, such mechanistic findings could be translated to primary endothelial cells isolated from patients with T2D (7). A recent study has indeed shown that PKCβ and NF-κB are highly activated in the diabetic endothelium and account for oxidative stress, decreased bioavailability of nitric oxide (NO) and impaired insulin signalling (7). Most importantly, such molecular changes correlated with endothelial dysfunction, as assessed by flow-mediated vasodilation of the brachial artery (7).

Mechanism-based therapies in T2D

Ruboxistaurin (RBX)

Many researchers around the globe have shown that disturbed PKCβ signalling is a major fingerprint of diabetes-related vascular phenotype, being responsible for structural and functional changes including alterations of cellular permeability, inflammation, angiogenesis, cell growth, extracellular matrix expansion and apoptosis (8). Understanding the regulation of this complex enzyme led to perception that its antagonism could be protective against CV complications in diabetes. Growing experimental evidence fostered the development of pharmacological inhibitors and clinical phase II-III trials to test the efficacy of PKCβ blockade in diabetic patients. In some studies, treatment with *Ruboxistaurin* (RBX), the most common used PKCβ inhibitor, prevented endothelium-dependent vasodilatation abnormalities in diabetic patients (9,10) whereas other evidence did not confirm these results (11). RBX is particularly effective in preventing diabetic retinopathy and macular edema, as shown in the PKC DRS2 trial (12). In 2006, the manufacturer Eli Lilly has received an approvable letter from the FDA for the prevention of vision loss in patients with diabetic retinopathy, but at this time the medication is not available for clinical use pending results of additional trials for this indication. Further studies are needed to determine whether RBX can effectively improve micro- and, most importantly, macrovascular complications in diabetic patients.

Metformin

Growing evidence in rats and mice indicate that metformin, a biguanide often used in the treatment of diabetes, has clear cardioprotective effects during ischemia and prevents adverse left ventricular remodelling in this setting (13). This effect is largely mediated by activation of AMP-activated protein kinase (AMPK), a key molecule orchestrating biochemical events such as glucose uptake, glycolysis, oxidation of free fatty acids (FFAs) and mitochondrial biogenesis (14). These processes significantly contribute to raise ATP levels and restore myocardial contractile efficiency. On this ground, researchers are now testing the cardioprotective effects of metformin in the clinical setting. A recent randomized trial, the GIPS III study, has postulated that metformin may improve left ventricular function following ST elevation myocardial infarction (STEMI) (15). Metformin (500 mg twice/day), administered 3 hours after percutaneous coronary intervention (PCI) failed to improve LV ejection fraction after 4-month follow-up. Different factors including follow-up duration, population risk as well as timing of metformin administration might have contributed to these negative results. Whether metformin may represent a cardioprotective agent in the clinical arena remains elusive and further randomized studies are warranted to clarify this issue.

PPAR α/γ agonists

The beneficial effects of dual peroxisome proliferator-activated receptorα/γ (*PPAR*α/γ) agonists reported in animal models of T2D led to the design of randomized trials to test their efficacy in diabetic patients (16). A phase II trial examining a novel dual *PPAR* α/γ agonist, aleglitazar, showed that therapy with this agent reduced hyperglycemia and favourably modified levels of HDL-C and triglycerides with an acceptable safety profile (16). However, in the recent AleCardio trial, aleglitazar was associated with a significant increase of renal and CV complications, leading to a premature interruption of study for safety concerns (17).

Therefore, evidence reported so far suggests that theoretical benefit and safety of dual *PPAR*-α/γ agonism have not been demonstrated to reduce macrovascular complications in patients with diabetes.

GLP-1 agonists and DPP-4 inhibitors

Novel therapeutic options for T2D based on the action of the incretin hormone glucagon-like peptide-1 (GLP-1)

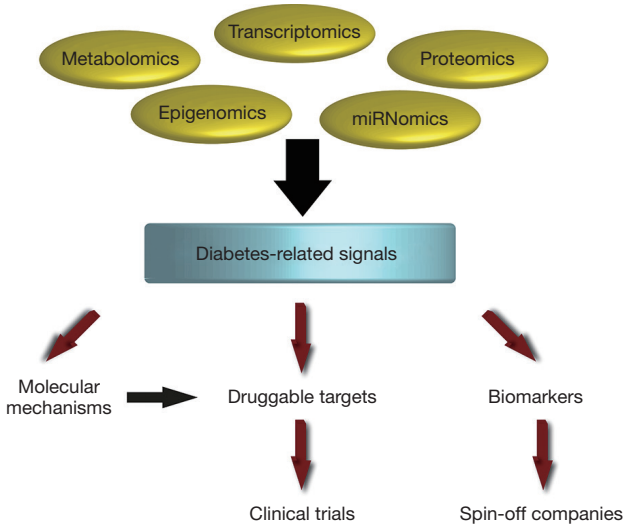

Figure 1 Schematic showing "omics" approaches employed to unveil diabetes-related signals and their implications in the development of mechanism-based approaches.

were introduced in 2005. Incretin-based therapies consist of injectable GLP-1 receptor agonists solely acting on the GLP-1 receptor and dipeptidyl-peptidase inhibitors (DPP-4 inhibitors) as oral medications raising endogenous GLP-1 and other hormone levels by inhibiting the degrading enzyme DPP-4. Despite promising results on intermediate vascular endpoints and inflammation, incretin therapies with GLP-1 agonists and DPP-4 inhibitors do not seem to improve CV outcomes on top of conventional anti-diabetic treatment, as recently shown in SAVOR TIMI-53 and EXTREME trials (18).

Need for novel biomarkers

One of the main evidence gaps in the management of DM consists in the stratification of CV risk. Available risk calculators remain flawed at several levels whereas reliable and cost-effective biomarkers are lacking (19). As of today, albuminuria is the only powerful predictor of incident CV events and heart failure in T2D patients. Atherosclerotic disease remains undiagnosed in many DM patients and accounts for high rates of myocardial infarction and stroke in this population. Therefore, the discovery of novel biomarkers is a major challenge in this area.

Despite the initial enthusiasm, the clinical utility of genetic biomarkers for prediction and prevention of atherosclerotic disease has proved to be limited. This is partially explained by the fact that environmental cues are emerging as a potent drivers of altered cardiac and vascular phenotypes during the life course (20). Environmental factors may trigger dynamic changes in gene expression and protein synthesis which occur regardless of DNA sequence, suggesting that postgenomic approaches may provide more detailed and timely snapshots of the etiological processes linking diabetes and CV disease (19,21). Indeed, we currently lack a detailed understanding of how gene and environment are interconnected in the natural history of atherosclerosis in people with cardiometabolic disturbances.

The "omics" world

More recently, high throughput "omics" technologies came on stage in the attempt of unmasking novel diabetes-related biological signals and their link with CV phenotypes (*Figure 1*) (22). This approach has generated considerable enthusiasm among scientists since it may provide a detailed characterization of disease processes fostering novel biomarkers and therapeutic strategies (23). The possibility to analyse thousands or more molecules simultaneously has given 'omics' the ability to generate enormous quantities of data which may somehow offer a precious "window on the disease". Signatures deriving from metabolomics, transcriptomics and proteomics are important to understand molecular networks and their interplay in the aetiology of diabetic atherosclerosis. Epigenomics is also key to understand altered expression of pro-atherosclerotic genes. This research area has exploded with the identification of plastic epigenetic changes of DNA/histone complexes which may critically regulate gene expression at the transcriptional level (24). The rapid growing of epigenetic research is outlined by PubMed search for "epigenetic and CV disease" showing that all available evidence comes from the last 15 years, and yielded to more than 1,300 peer-reviewed articles published (24). The possibility that DNA and chromatin alterations may contribute to diabetes-related phenotypes will be strongly supported by large-scale initiatives such as the International Human Epigenome Consortium, aimed at mapping 1,000 reference epigenomes within a decade (25). Such wide epigenomic analysis will be instrumental for the identification of epigenetic variations specifically associated with major pathological states including T2D and CVD. Data obtained with both site-specific and epigenome wide approaches are contributing to the identification of specific cytosines in the human genome where either addition or removal of an electrophilic methyl

group is significantly associated with altered transcription of oxidant and inflammatory genes involved in diabetes-related vascular complications (26). Albeit omics might foster attractive discoveries, such "fishing expeditions" are usually very expensive and not always productive, especially when methodology and cohort selection are not appropriate. Molecular profiles generated by the use of omics technologies can be very sensitive to specimen collection, processing and storage conditions. The main problem when it comes to omics is represented by their reliability. In this regard, the US National Cancer Institute (NCI) has recently stated that few omics-based predictors have been translated successfully into clinically useful tests, and the difficulty in clinical translation is mostly represented by issues concerning accuracy of omics-based test for definitive evaluation in a clinical trial (27). However, omics approaches may lead to important advances in CV risk stratification in patients with DM. Amino acids profiling in plasma samples using liquid chromatography-tandem mass spectrometry revealed that branched-chain and aromatic amino acids were strongly associated to incident CVD [HR =2.20 (range, 1.12-4.31) for upper quartile] and atherosclerosis, as assessed by carotid intima media thickness (CIMT, P=0.035) (28). Moreover, micro-RNA analyses have recently unmasked promising biomarkers of diabetic vascular disease. The level of circulating miR-126 was found to be decreased in a glucose-dependent fashion in T2D patients, resulting in impaired vascular endothelial growth factor (VEGF) facilitation (29). A down-regulated blood level of miR-126 was also demonstrated in CAD patients (30). However, further studies are warranted to confirm the biomarker potential of miR-126 in the setting of T2D.

The lab to market agenda: where do we stand?

It is not always the case that promising technologies are transferred from the laboratory to the marketplace. Different factors may significantly delay the implementation of a "lab to market agenda". This might happen, for example, when mechanistic discoveries are translated too early, without exhaustive molecular definitions. Another fall in the system is represented by design and methodology of preclinical and clinical studies which are not always capable to unmask potential benefits of emerging therapeutic or diagnostic technologies. Furthermore, the issue of funding may significantly affect the clinical applicability of experimental research. In this regard, the EU investment in Research and Innovation has progressively increased, with 10.84 billion euro spent in 2013 as compared to 5.48 in 2007 and 7.54 in 2010 (31). Science and Health sectors are being highly prioritized with a cumulative expenditure of 2.71 billion euro only in 2013. Similarly, the US Federal Government will spend more than $130 billion on research and development in 2015 (32). Undoubtedly, such efforts will significantly contribute to foster basic research programs and accelerate promising technologies from the laboratory to the marketplace.

Conclusions

Despite clear difficulties in the applicability of research breakthroughs, translational research initiatives remain the most powerful strategy to promote novel mechanism-based therapeutic strategies and biomarkers in people with T2D. The understanding and interpretation of novel biological signals will promote knowledge and efforts to develop future strategies to fight CV disease burden in diabetic patients.

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Footnote

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