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Editorial

The discovery of PCSK9 inhibitors: A tale of creativity and multifaceted translational research

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> Creativity is defined as producing ostensibly out of nothing, something of Beauty, Order or Significance, *Peter Medawar (1915–1987)*
> Creativity is just connecting the dots, *Steve Jobs (1955–2011)*

Atherosclerosis, obesity, and metabolic syndrome are closely linked and constitute, arguably, the most menacing three conditions to modern society. Thus urgent, concerted efforts at several levels, utilising modern tools, are required to tackle them. Translational research is a rapidly expanding branch of science, dedicated to rapid delivery of discoveries from the bench to the bedside. The growing importance of this specialty in our field is evidenced by the establishment of the International Society of Cardiovascular Translational Research and its dedicated Journal by Nabil Dib and colleagues.[1]

The fundamental discovery of the relationship between, lipids, cholesterol and atherosclerosis was made in the 1960s.[2–4] This led to the discovery of lipid lowering drugs, including statins, which constitute one of the blockbuster drugs of modern times. In spite of that, the toll of the three conditions mentioned above continued almost unabated, particularly in patients with familial hypercholesterolemia (FH) in whom LDL apheresis and/or liver transplantation are needed in many instances to achieve adequate reduction of LDL-cholesterol levels.[5,6] The landmark discovery of the role of the LDL receptors in lipid clearance by Goldstein and Brown (Figures 1 and 2)[7,8] earned them the Nobel Prize, and opened the door to further translational research which led to several further discoveries, including that of Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9). In addition to establishing the role of the LDL receptors in clearing LDL-C from the circulation, and that FH is due to a mutation in the LDL receptor gene, Brown and Goldstein described three new cellular processes, including receptor-mediated endocytosis (paving the way for further research into the mechanisms of vesicle transport and cellular trafficking; work for which Rothman, Schekman and Sudhof have recently been awarded the Nobel Prize in Medicine), receptor recycling, and feedback regulation of receptors, which led to discovery of hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase and statins.

Several years later, the discovery of a major regulatory pathway controlling the number and function of the LDL receptors was made by a large number of researchers working in different

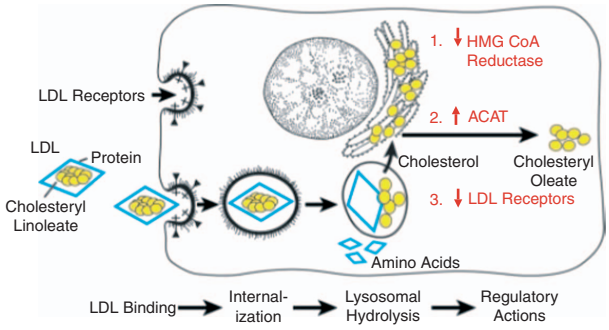

Figure 1. LDL receptor pathway of mammalian cells *(from Goldstein J and Brown S. The LDL Receptor. Arterioscler Thromb Vasc Biol. 2009;29:431–438).*

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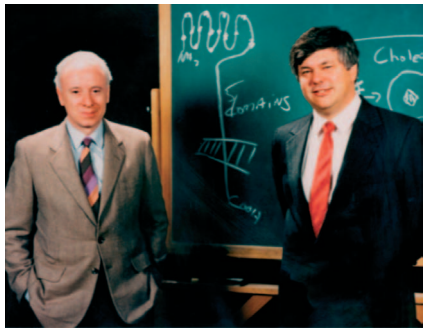

Figure 2. Joseph L. Goldstein and Michael S. Brown.

fields of translational research. This was started by the massive contribution of Nabil Seidah's group from Montreal (Figure 3). While working on the chemistry and functions of a family of proteins termed proprotein convertases, they identified PCSK9 encoded by a gene on chromosome 1 (Figure 4). Collaborative work with a French group showed that a gain of function mutation in that gene was responsible for FH in a French family.[9] This was followed by similar findings from Oslo.[10]

Figure 3. Nabil Seidah.

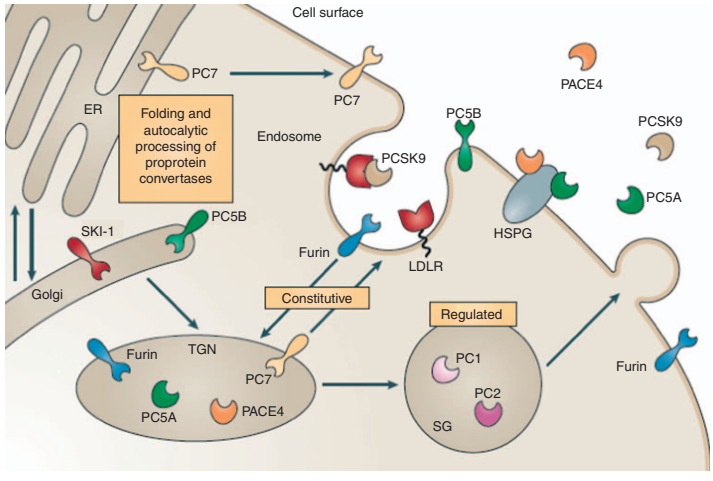

Figure 4. Subcellular localization of proprotein convertases. Upon exiting the endoplasmic reticulum (ER), most of the basic amino acid-specific proprotein convertases traverse the Golgi apparatus towards the *trans*-Golgi network (TGN). The activated membrane-bound subtilisin kexin isozyme 1 (SKI1) is mostly concentrated in the *cis*- and *medial*-Golgi, from where it is then sent to lysosomes for degradation, and does not normally reach the cell surface. Proprotein convertase subtilisin kexin 9 (PCSK9) is secreted from the TGN directly into the medium as an enzymatically inactive non-covalent complex of the protease and its prosegment. Upon binding to the low-density lipoprotein receptor (LDLR) at the cell surface, the PCSK9–LDLR complex is internalized into endosomes and then sent to lysosomes for degradation. *(from Seidah N and Prat A. The biology and therapeutic targeting of proprotein convertases. Nat Rev Drug Discov. 2012 May;11(5):367–83).*

In contrast, findings from the Dallas Heart Study, showed that loss of function mutations in PCSK9 gene in a subset of Afro-Americans were associated with very low cholesterol levels and markedly reduced incidence of cardiovascular disease.[11] The mechanisms involved are schematically shown in Figure 5.

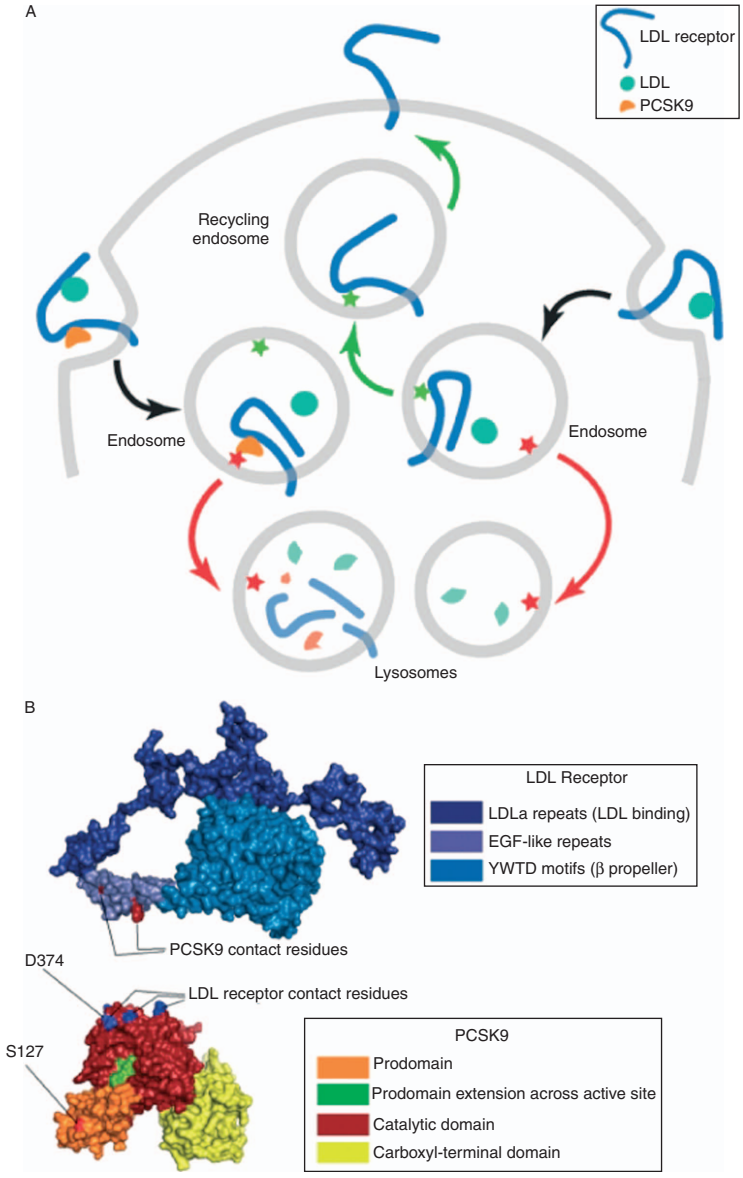

Figure 5. A: Model of PCSK9-mediated sorting of LDL receptors to lysosomes. The EGFa domain of the LDL receptor is required for proper sorting of the LDL receptor back to the cell surface. The EGFa domain may contain a sorting signal that interacts with an endosomal protein (green star), directing the LDL receptor back to the cell surface on recycling endosomes (green arrows). Binding of PCSK9 might interfere with that signal, preventing the LDL receptor from returning to the cell surface. Alternatively, PCSK9 could contain a distinct sorting signal (red star) that results in the sorting of the PCSK9-LDL receptor complex (red arrows) to lysosomes. The gain-of-function mutation involving S127 of PCSK9 may enhance the sorting of the PCSK9-LDL receptor complex to lysosomes. B: The structure of the LDL receptor and PCSK9 at endosomal pH. The LDL receptor is folded back upon itself at low pH; however, the face of the EGFa domain that binds PCSK9 is exposed. The LDL receptor-binding site on PCSK9 is at the apex of a roughly triangular structure formed by the tripartite domain structure of PCSK9. The D374 residue that is altered in gain-of-function PCSK mutants is located within the apical LDL receptor-binding site, whereas the S127 residue is quite distant from the binding interface. S127 mutations do not affect binding of PCSK9 to the LDL receptor. Gain-of-function mutations affecting residue 127 may reduce LDL receptors by enhancing the sorting of LDL receptors to lysosomes, rather than by affecting the strength of PCSK9-LDL receptor interactions. *(figure and accompanying legend from Peterson A, Fong L and Young S. PCSK9 function and Physiology. J Lipid Res. 2008 June; 49(6): 1152–1156).*

THE RUSH TO THE CLINIC

Not surprisingly, the above mentioned exciting findings was followed by the development of several strategies to inhibit the protein or RNA using monoclonal antibodies (Figure 6) or RNA interference drugs respectively,[12–19] with extremely promising results in Phase 1 and 2 trials. Inhibition of PCSK9-mediated degradation of LDL receptors by an epidermal growth factor-like repeat A (EGF-A) peptide has also been demonstrated in a mouse model.[20]

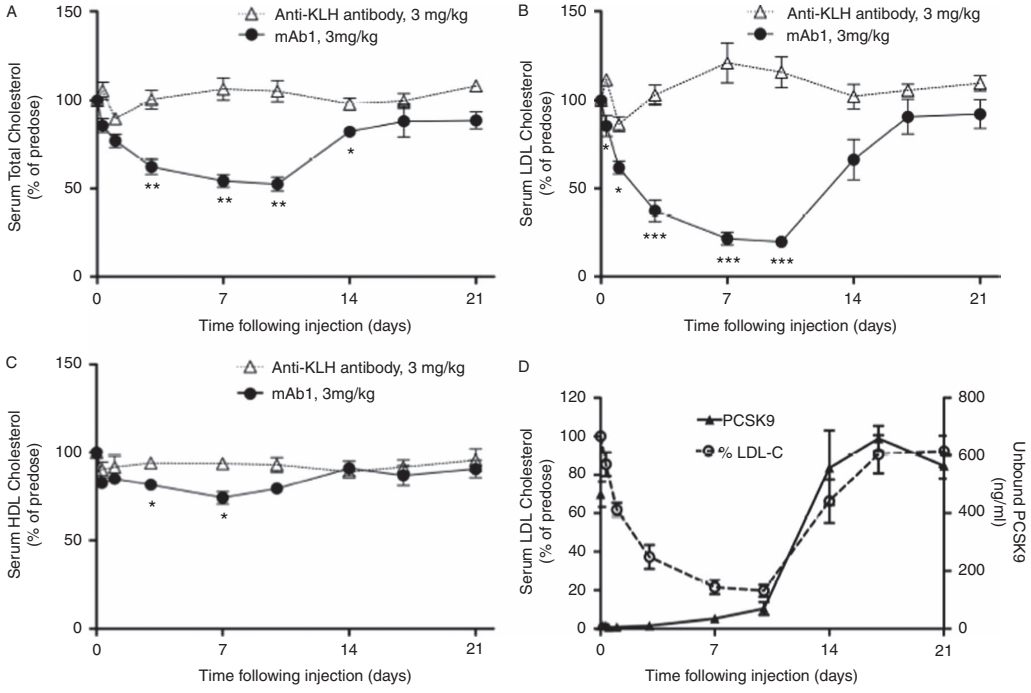

Figure 6. Total cholesterol, LDL-C and HDL-C levels after injection of a neutralizing monoclonal antibody against PCSK9 compared to a control antibody against keyhole limpet hemocyanin (KLH). Results are expressed as mean ± SEM. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ vs. anti-KLH control antibody at the same time point, n = 4 per group. ***(from Chan J. et al. A proprotein convertase subtilisin/kexin type 9 neutralizing antibody reduces serum cholesterol in mice and nonhuman primates. Proc Natl Acad Sci U S A. 2009 Jun 16;106(24):9820–5).***

THE FUTURE

The search for other means of inhibiting PCSK9 continues. Increased expression of PCSK9 has recently been shown to be a key mechanism by which human resistin – an adipose tissue-derived adipokine – downregulates hepatocyte LDL receptor expression,[21] hence increasing the risk of atherosclerotic cardiovascular disease.[22,23] This interesting link opens the door for future research into resistin inhibition as another strategy for inhibiting PCSK9; possibly with added pleotropic effects.[24]
Such drugs are expected to have a major effect on cardiovascular health, and represent a triumph for translational research. The story stands as a prime example of creativity as defined by the late Sir Peter Medawar, with a methodology that follows Steve Jobs' approach.[25]

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