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Therapeutic targeting of cell transition: ready for clinical prime-time?

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Atherosclerosis, characterized by the build-up of lipid-laden plaques, is the main underlying cause of various cardiovascular diseases (CVDs), including myocardial infarction, peripheral vascular disease, arterial calcification, ischaemic cardiomyopathy, and a significant proportion of strokes. Endothelial cells, lining the luminal surface of the vessels, are major regulators of vascular homoeostasis and thereby play a crucial role in the development of atherosclerosis. Activation of endothelial cells by atherosclerotic risk factors results in cellular dysfunction and in the expression of pro-inflammatory cytokines and adhesion molecules, driving leukocyte migration and promoting the accumulation of leukocytes in the intima of the artery.

Over the last two decades, numerous studies have demonstrated that endothelial cells can acquire mesenchymal cell-like properties in a process called endothelial-to-mesenchymal transition (EndMT). During this transition, endothelial cells lose expression of classical cell markers, like VE-Cadherin and CD31, while they gain expression of mesenchymal markers, like alpha-smooth muscle actin. Through these cellular changes, EndMT contributes to CVDs like atherosclerosis[1,2] and to other processes and diseases including the vascular remodelling and neointima formation following vein grant transplantation.[3] Although the exact role and underlying mechanisms of EndMT in atherosclerosis and other CVDs are being rigorously investigated at the moment, so far there still remain large knowledge gaps.

The endothelium is a specialized type of epithelium. Just as endothelial cells can undergo EndMT, epithelial cells can also undergo epithelial-to-mesenchymal transition (EMT). As it happens, there is a far larger body of knowledge relating to EMT than EndMT, with EMT first formally described in the 1960s.[4,5] Therefore, many lessons about EndMT can potentially be learned from the EMT process. Physiologically, EMT plays an important role in embryogenesis and wound healing, but on a pathological level, it has already been well established that EMT contributes to malignant progression, for example by increasing metastatic potential.[6] Since carcinomas originate in epithelial tissues, EMT is understood to play a particularly important role in this type of cancer. The likely mechanism(s) whereby EMT promotes malignancy of epithelial tumours is by the acquisition of hallmark features of mesenchymal cells including increased motility, loss of cell–cell adhesion, and detachment from the epithelial basement membrane. Due to its important role in carcinoma progression, it has been proposed that the inhibition of EMT may be a promising therapeutic oncological approach, although due to several limitations, concrete evidence for this clinical potential has remained elusive so far.

In a recent publication in *Nature*, Cassier *et al.*[7] in their study *Netrin-1 blockade inhibits tumour growth and EMT features in endometrial cancer* show clear pre-clinical and clinical evidence that EMT inhibition indeed has great clinical potential. Focusing on endometrial adenocarcinomas, the authors were able to show that netrin-1 and its main receptor UNC5B are up-regulated in tumour tissue compared with normal endometrium. Based on this, Cassier *et al.* developed a monoclonal antibody called NP137 that neutralizes netrin-1 and blocks the netrin-1–UNC5B interaction. Treatment of mice with NP137 significantly decreased the development of endometrial tumours and increased the survival rate of these mice, demonstrating its potency. The authors then moved to study NP137 in patients with advanced endometrial carcinoma. In a Phase 1 trial with 14 patients who received NP137 every 2 weeks, there was no dose-limiting toxicity and 8 out of 14 patients achieved disease control (stable disease). Furthermore, at least in one exemplary case, NP137 treatment resulted in a striking 51% reduction of liver lesions within 6 weeks (*Figure 1*).

Interestingly, the primary mechanism behind these effects of NP137 was found to be inhibition of EMT, characterized by a decreased expression of mesenchymal genes and increased expression of the epithelial marker EpCAM. Inhibition of EMT occurred not only in mice but also in patients treated with NP137. Furthermore, single-cell RNA sequencing of lung biopsies before and after treatment showed that NP137 significantly reduced the tumour cell compartment. Besides this net decrease in cancer cells, the EMT score was also strongly decreased, demonstrating a more epithelial phenotype. It has already been described that EMT is a major cause of chemotherapy resistance.[8] Since NP137 inhibits EMT, Cassier *et al.* also investigated whether treatment resulted in a more beneficial outcome by comparing carboplatin–paclitaxel (CarboTaxol), the standard-of-care chemotherapy for the type of malignancy investigated, alone or with addition of NP137. At least in mice, it could be clearly demonstrated that the combined NP137/CarboTaxol treatment was superior to CarboTaxol alone, thus creating the possibility that additive NP137 (on top of other agents) may hold promise for overcoming chemotherapeutic resistance. This notion is currently being further investigated in the Phase 2 GYNET trial (NCT04652076) in patients with endometrial or cervical cancer.

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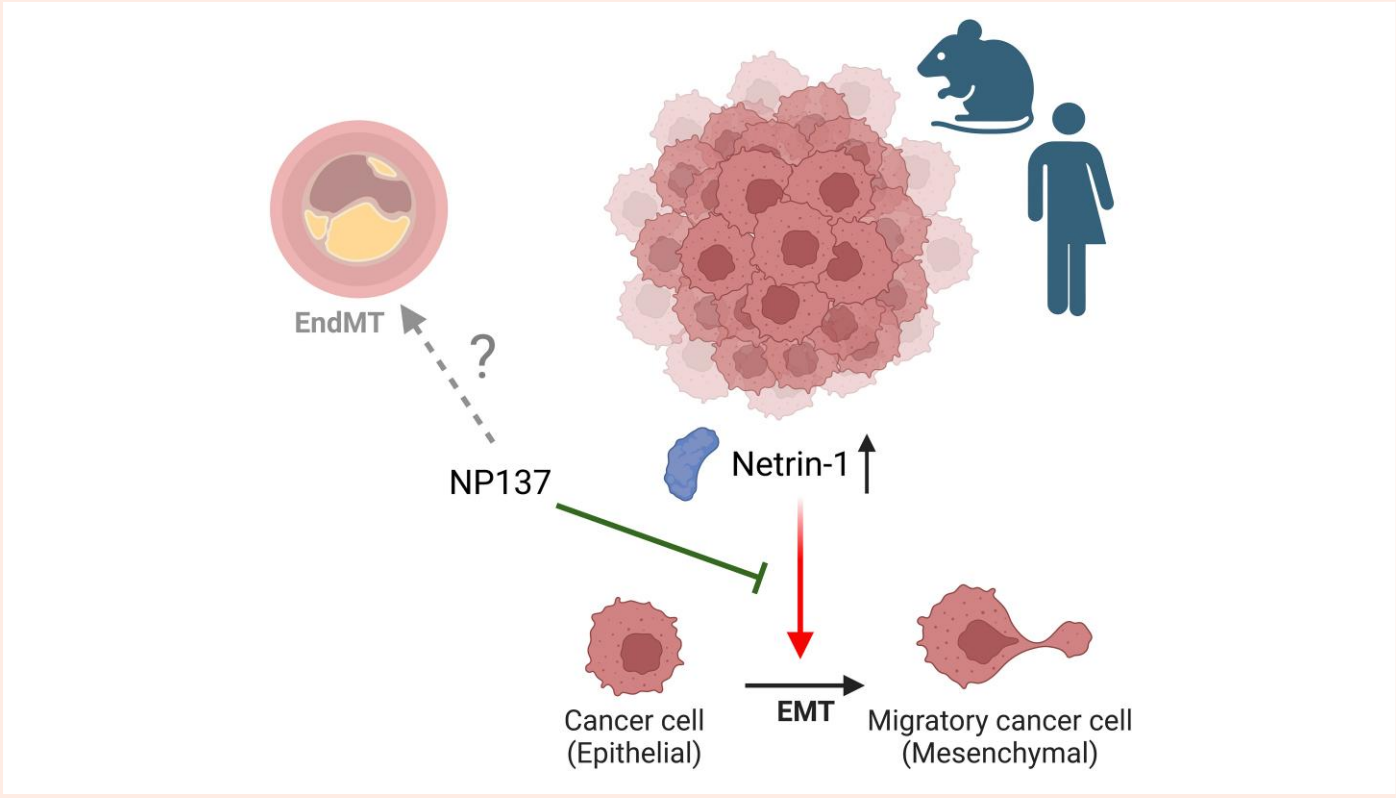

Figure 1 EMT targeting using NP137. The study by Cassier *et al.* demonstrated in mice and humans that netrin-1 is up-regulated in endometrial carcinomas. Blocking of netrin-1 by the administration of NP137 led to decreased EMT and reduced tumour progression, validating its clinical potential. Future research should focus on the effects of NP137 in a cardiovascular setting, for example by influencing EndMT. EMT, epithelial-to-mesenchymal transition; EndMT, endothelial-to-mesenchymal transition.

Although great progress has been made over the last years to understand the role of EMT in tumours, so far no pharmacological interventions are available to specifically target EMT in the clinic. Therefore, this study is seminal by proving the clinical potency of NP137. Although the cancer research field is leading the way in efforts to bring the targeting of EMT to the clinic, it is important to extrapolate such observations to other research fields like CVD. For example, intense research efforts have been invested in the identification of compounds that could be used as EndMT inhibitors. Although many of these studies are pre-clinical in nature, several promising approaches have already been identified.[9] However, a number of hurdles remain to be overcome, including a lack of robust human data related to EndMT and thus translational proof, and the lack of a standardized definition of EndMT. Furthermore, systemic targeting of EndMT as a therapeutic approach, say for example as a potential approach to stabilize atherosclerotic plaques, would require very careful evaluation due to the likely need for prolonged administration and thus off-target effects. For example, in relation to the current study where EMT is targeted by blocking netrin-1, major side effects may occur in a CVD context as netrin-1 itself has cardioprotective effects after ischaemia–reperfusion injury following a myocardial infarction, which may be lost after netrin-1 blocking approaches.[8] Therefore, an approach whereby local delivery could be achieved might be favourable, though still very challenging from a technical perspective.[10] Nevertheless, efforts are being made, particularly in pre-clinical studies, to direct nanoparticles to endothelial cells or macrophages, by adding specific peptides to the surface of the particles enabling cell-specific homing. Although such approaches are still in their infancy, it could be speculated that such local delivery methods would also be beneficial in the context of targeting EndMT in atherosclerosis and potentially for other CVDs.

Ultimately, this study from Cassier *et al.* highlights a long-awaited shift in our outlook towards EMT—being from an interesting biological 'side observation' or disease 'epiphenomenon', to EMT now being central to disease pathogenesis and a main therapeutic target. While the CVD field is still seemingly years behind that of our oncological colleagues, seen optimistically, this study provides important rationale and motivation to suggest that the targeting of EndMT to ameliorate various CVDs might also someday evolve into an important and unique therapeutic approach.

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Data availability

The manuscript does not contain any new data.

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Biography: Dr Emiel van der Vorst obtained his PhD in Cardiovascular Biology and Medicine from the Cardiovascular Research Institute Maastricht (CARIM), The Netherlands in 2015, by studying the effects of high-density lipoproteins on inflammation. For his post-doctoral period, he investigated the role of chemokines and chemokine receptors in atherosclerosis at the Institute for Cardiovascular Prevention (IPEK) in Munich, Germany, resulting in several high-impact publications. Since 2019, he has been working as group leader at the Institute for Molecular Cardiovascular Research (IMCAR) in Aachen, Germany, as well as at CARIM and IPEK. Currently, his research is focusing on elucidating various mechanisms by which lipid metabolism interacts with the immune system in the context of cardiovascular disease, especially atherosclerosis. His work is funded by various prestigious (inter)national personal grants and he is member of the editorial boards of several cardiovascular focused journals. Furthermore, he is a nucleus member of the Scientists of Tomorrow from the European Society of Cardiology.

Biography: Professor Jason Kovacic graduated from The University of Melbourne Medical School in 1994 and then undertook cardiology training at St Vincent's Hospital in Sydney including a PhD in cardiovascular medicine at the Victor Chang Cardiac Research Institute. In 2007, he relocated to the USA, initially to do a post-doc at the National Heart, Lung and Blood Institute (NHLBI) at the National Institutes of Health (NIH) in Bethesda, Maryland. After this post-doc, he moved to The Icahn School of Medicine at Mount Sinai in New York. In early 2020, Jason was appointed as the Institute Director and CEO of the Victor Chang Cardiac Research Institute in Sydney, Australia. He continues to practice as a clinical cardiologist and runs a research programme focused on atherosclerosis, EndMT, fibromuscular dysplasia, and other vascular diseases.