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A mucin degrader for cancer therapy

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A new approach to targeting mucins via protein degradation shows promise against cancer.

Mucins — barrier glycoproteins that form mucus and other gel-like secretions — have long been a target of interest in oncology, but their thick coating of sugars and diverse functions have defied traditional drug discovery. Although multiple drug candidates targeting mucins have been tested in clinical trials, none has shown compelling clinical benefit. Writing in Nature Biotechnology, Pedram et al.[1] now propose a new drug modality to target cancer-associated mucins: an engineered mucin-degrading enzyme linked to an antibody against a cell-surface marker on cancer cells. Using a variety of *in vitro* and *in vivo* models, the authors demonstrate that the approach shows promise in breast cancer, meriting further pre-clinical investigation.

Mucins are defined as glycoproteins that have >50% of their mass as carbohydrate. They are expressed on most epithelial cells (cells that line the ducts in the body) and are predominantly, but not exclusively, over-expressed in the carcinomas that arise from this cell type[2]. Many mucins are considered oncogenes, and they exert their pro-tumorigenic effects in several ways. First, they can present a barrier around the cancer cell, preventing immune eradication and facilitating electrostatically mediated spread[3]. Second, through their intracellular signalling motif, membrane mucins can signal after interacting with cognate ligands, lectins, bacteria and viruses or after cleavage by several common proteases, leading to activation of pro-proliferative and pro-survival pathways in epithelial cells[4]. Third, through their sugars, they can interact directly with lectins carried on immune cells, resulting in modulation that supports tumor survival, growth and spread[5]. Finally, they can promote metastasis, either through direct binding of their carbohydrates to receptors elsewhere in the body (e.g., sLe^x binding E-selectin, or core 1 binding galectin-3), or indirectly by enhancing the function of adhesion molecules[6,7].

It is important to note that the increased expression of mucins commonly seen in cancer arises not from genetic mutations or gene amplification but from local inflammatory factors released in the tumor microenvironment. Indeed, the growing consensus is that mucins, beyond their protective and biome-orientated functions, support healthy epithelial and epithelial-immune homeostasis by regulating re-epithelialisation and local immunity, with dysregulation of these processes seen in diseases of chronic epithelial inflammation, such as carcinomas and interstitial lung disease. As such, targeting mucins for cancer therapy is of interest not only because they are biomarkers of disease but also because they have a pathological role.

A particularly popular target is the MUC1 mucin[8]. Most therapeutic approaches have focused on the MUC1 extracellular domain, targeting it, for example, with antibodies to deliver toxins and radioisotopes or with chimeric antigen receptor-T cells. Approaches directed to the cytoplasmic tail, aimed at inhibiting signalling, are also being investigated. The study by Pedram et al. describes an entirely new strategy that is applicable to most members of the mucin and mucin-like family. Here the aim is to deliver a bacterial mucin-degrading enzyme, a mucinase, capable of degrading any protein carrying a 'mucin motif' at the surface of cancer cells. In its reliance on protein degradation, the approach resembles proteolysis-targeting chimeras (PROTACs)[9]. However, PROTACs induce intracellular proteolysis in the proteasome, whereas Pedram et al. use a protease against extracellular proteins.

To increase specificity toward cancer cells, the authors fuse the bacterial mucinase StcE to an antibody against HER2, a receptor commonly over-expressed by some cancers and the target of the breast-cancer drug trastuzumab (Herceptin). There are two important aspects of this construct. First, engineering of the mucinase is used to lower its activity, ensuring that it is active only when concentrated at the cell surface. Second, the targeting antibody is a nanobody, an antibody is derived from the camel family that contains only a heavy chain. Importantly, this means the antibody cannot bind immune cells, preventing 'mopping up' of the construct and unwanted bystander effects.

Tests in mixed cell cultures show that the construct effectively degrades the mucins on a HER2-expressing cell line but not on a non-expressing line. These experiments

demonstrate the absolute requirement for an engineered low-activity mucinase, as the wild-type enzyme construct degrades mucins on both HER2-expressing and non-expressing lines. Further *in vitro* experiments show that removal of mucin by eStcE-HER2 enhances natural killer cell killing and macrophage phagocytosis of $HER2^+$ cells. In a mouse model, the lungs of BALB/cJ mice were seeded by intravenous injection with a mouse mammary carcinoma cell line (4T07 cells) transfected with human MUC1, without the cytoplasmic tail, and human HER2. In this *in vivo* model, eStcE-HER2 limits tumor growth and metastasis while also altering the local immune environment to a more 'anti-tumor' state without any obvious toxicity to the animals (**Fig. 1**).

This study is an extremely encouraging start to the use of targeted mucinases for cancer therapy. However, as is often the case, more pre-clinical work is needed before the approach can be taken forward into the clinic. As mentioned, mucins such as MUC1 can drive tumor growth and survival through signalling after ligation or cleavage via their intracellular domain. Quite correctly, Pedram et al. use models in which MUC1 lacks this intracellular domain so as to remove a variable and aid interpretation. However, this means that we do not yet know the impact of the treatment on cancer growth or survival through this mechanism. The use of human transgenic models for mouse studies will also be an important next step. Although mouse and human HER2 have a high level of sequence identity, many antibodies, such as trastuzumab, do not cross-react with mouse Her2 (ref. 10). This is important as HER2 is known to be expressed at lower levels by some normal tissues and cells, including cardiomyocytes, leading to toxicity when targeted.

A more general hurdle to be overcome is the fact that mucin production by cancers is a continuous process driven by local inflammation, meaning that continuous treatment would be needed until a satisfactory clinical endpoint is reached. Other targeted therapies also require continuous treatment, but in this case the use of a bacterial, non-self protein would likely induce increasing anti-drug immunity over time, preventing long-term administration. However, the authors' data demonstrate that a short treatment regime may indeed be sufficient to tip the immunological balance towards tumor eradication.

Finally, the authors suggest that their approach may be applicable to other diseases that over-express mucins, such as cystic fibrosis and gut dysbiosis. However, these two conditions occur in organs where the mucin layer is crucial for protection or microbiome function, so caution will be required in developing such applications. Overall, the work of Pedram et al. is an exciting new approach toward an intractable target, with great potential in cancer therapy.

References

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Competing interests

The authors declare no competing interest

Figure Legend

Fig 1: **Action of the HER2 targeted mucinase (αHER2-eStcE) and the resultant change in immunological landscape.** The mucins and mucin-like proteins expressed by $HER2^+$ cancer cells are degraded by αHER-eStcE. The mucinase has been engineered to have low activity and so is only active when concentrated at the tumor cell via targeting of HER2, ensuring specificity. Removal of the mucins and mucin-like proteins results in a change of immune cells surrounding the tumor in an *in vivo* breast cancer model, leading to slower primary tumor growth and reduced lung metastases.