Editorial

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Joining the dots – NEDDylation in cancer cells regulates the tumour environment in cholangiocarcinoma

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Solid tumours are a highly complex environment in which a multitude of cell types interact to promote disease progression and evade therapeutic intervention. Whilst much of the work investigating cancer has concentrated on the tumour cells themselves, understanding the role of the tumour microenvironment has gained increasing traction over the last decade and targeting this component of the tumour has been subject to renewed vigour, particularly in those cancers where therapies developed to clear tumour cells have had marginal impact.

The presence of a complex stroma, the fibrogenic, endothelial and immunological support structure of a tumour, is a hallmark of cholangiocarcinoma (CCA) where non-cancer cells can comprise up to 70% of the tumour mass.[1] The presence of this complex stroma is thought to contribute to the highly aggressive nature of this cancer and contributes to poor survival (~5% over 5 years) following diagnosis and wide metastatic dispersal in advanced disease.[2]

Increasingly, the cellular and non-cellular components of the CCA stroma are being defined, but our understanding of the crosstalk between tumour cells and the fibro-immune microenvironment in CCA is lacking. In both premalignant bile duct diseases (including those that increase the risk of CCA, such as primary sclerosing cholangitis) and in CCA itself, work from a range of laboratories has demonstrated that the biliary epithelium can communicate out to its microenvironment to regulate immune cell recruitment,[3] fibroblast activation[4] and directly influence the deposition of a new "proliferative" extracellular matrix by indirectly communicating with non-epithelial cells.[5] Similarly, the formation of the regenerative and cancer niche promotes progenitor traits in both biliary epithelial cells and their cancerous counterparts and likely enables both their proliferation during disease and also promotes their immune evasion.[6,7]

In order to better understand how cancer cells communicate with their tumour stroma, we not only need to understand the signals that are produced by the cancerous epithelium in CCA and define how these communicate with the local environment, but we must also determine what intracellular processes are occurring in the cancer cells themselves to facilitate tumour-stroma crosstalk. This is not an easy question to address, as the cancer proteome is subject to dynamic regulation at both the translational and post-translational levels.[8,9]

In this issue of *Journal of Hepatology*, Olaizola and colleagues define how NEDDylation – a post-translational modification where NEDD8 (a ubiquitin-like molecule) is conjugated to its target proteins – regulates tumour progression[10] (Fig. 1). NEDDylation of a protein often serves to alter the target proteins ability to interact with co-factors or to form complexes with other proteins, thereby affecting multi-protein complex assembly and function. In this report, Olaizola *et al.* found that, in healthy patient liver tissues, expression of the NEDDylation machinery is low, but in both premalignant (BilINs) and malignant tissue, expression of the NEDD8-specific E1 ligase, NAE1, which is essential for the conjugation of NEDD8 to proteins, is increased in human disease. Critically, when examined further, the authors identify a range of NEDD8-conjugated proteins that are associated with multiple oncogenic processes. The reliance of cancer cells on NEDDylation has been described in other, non-solid and solid malignancies and small molecules have been developed to limit the ability of NAE1 to add NEDD8 to its substrates. For example, pevonedistat is currently in a phase I clinical trial[11] and acts as a competitive AMP mimetic, thereby limiting the ability of NEDD8-AMP intermediates to form, which is a rate limiting step for NEDDlyation of NAE1 substrates. Olaizola and colleagues make use of the addiction that CCA cells have to protein NEDDlyation and demonstrate when treating human CCA cells *in vitro* or in xenografts in immunocompromised mice that pevonedistat reduces tumour cell proliferation and tumour progression. While a stalwart model in the field, xenografts do have their limitations and further work will be needed to really understand the interactions between CCA tumour cells and their microenvironment. A number of stroma-targeting approaches are being trialled in CCA and combining pevonedistat with either

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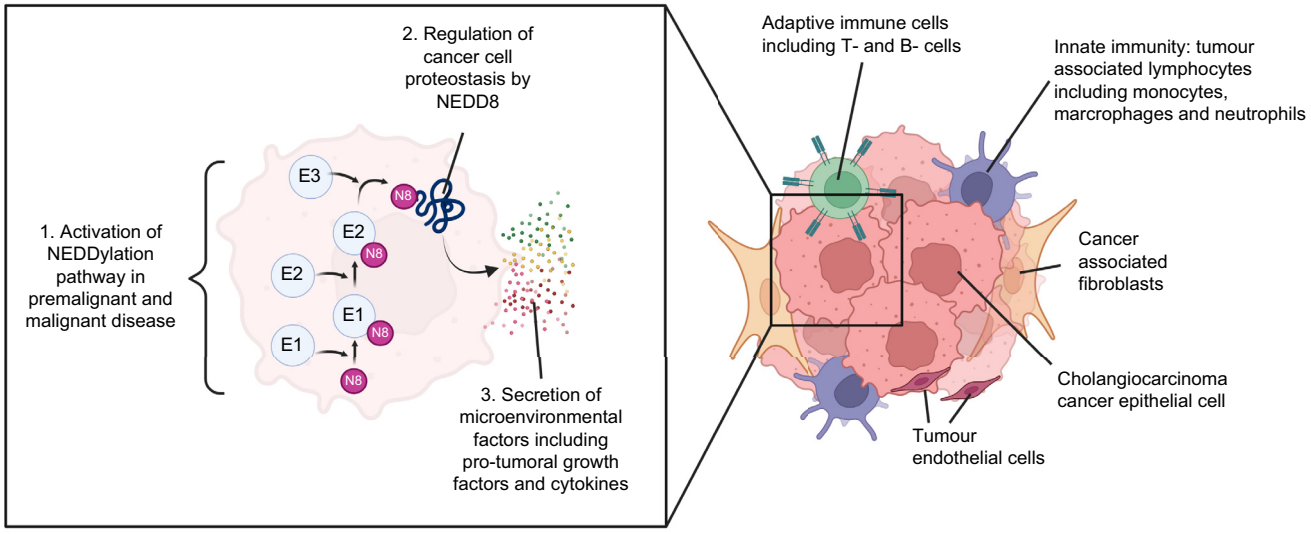

Fig. 1. Tumour cells are part of a complex organ comprising tumour cells and supporting cells such as cancer-associated fibroblasts, endothelial cells and immune cells. To generate this pathological tissue, cancer cells must alter their proteome and secretome to influence the microenvironment. Part of this regulation occurs through NEDDylation, a process by which proteins are post-translationally modified to alter their stability and ability to form biological protein complexes.

anti-PD-1/anti-PD-L1 immunotherapy[12,13] or anti-VEGF therapies such as regorafenib[14,15] in immunocompetent models could enhance uncoupling of cancer cells from their supportive stroma and further drive tumour regression (Fig. 1).

Whilst these findings are exciting, understanding the fundamental mechanism behind pevonedistat-reduced NEDDylation remains a challenge, as systemic therapies would alter these modifications in both cancer cells and those cells in the stroma that rely on NEDD8 function. To overcome this limitation, the authors make use of a sophisticated approach where they use a genetically modified mouse line in which NAE1 can be specifically deleted in tumour cells. When these animals are given CCA the tumours that form are smaller and have an altered secretome, where many families of pro-cancerous signals including Wnt, TNF and TGFβ are reduced, whilst apoptosis and necrosis are increased. By understanding how NEDDylation regulates cancer cell-stromal interactions, Olaizola *et al.* have opened a window into the complexity of the tumour organ in CCA.

Over recent years, the development of precision medicine approaches in CCA have expanded the therapeutic toolkit. The reality remains, however, that even with these targeted therapies (including FGFR[16] and IDH1[17]-inhibitors) the majority of patients do not carry therapeutically actionable mutations and subsequently the majority receive chemotherapy as standard of care. With advances such as the one described here by Olaizola *et al.*, where pevonedistat can be used to disrupt a fundamental biological function on which cancer cells rely, there is the potential for NEDDylation-inhibitors to be a broad-acting therapeutic in CCA regardless of the underlying genetic status or anatomical location. Furthermore, it is clear that there are groups of patients, such as those diagnosed with primary sclerosing cholangitis[18,19] (or other pre-malignant diseases including Caroli disease[20]) who live with a substantially increased risk of developing CCA. For these patients, extended long-term clinical monitoring is essential and the identification of prophylactic therapies that could limit the conversion of diseased ducts into cancer would be a substantial addition to their care. As Olaizola *et al.* demonstrated, the molecular machinery required for NEDDylation is upregulated in the pre-malignant disease state and, as such, pevonedistat has potential in this setting.

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Conflict of interest
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