Combination PARP and HDAC inhibition as a therapeutic strategy targeting liver cancer stem cells?

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Advances in imaging, surgery and medical therapy over the last few decades have resulted in steadily-declining cancer mortality rates across the globe. Mortality attributed to primary liver cancer, however, continues to rise (1). Liver cancer is responsible for over 700,000 deaths per year and is the second highest cause of cancer-related deaths worldwide (2).

Hepatocellular carcinoma (HCC) is the commonest primary liver cancer and geographical variations in HCC incidence and mortality largely reflect the prevalence of hepatitis B and C viral infections, which predispose to chronic liver disease (CLD) and HCC. In countries where the prevalence of viral hepatitis is low, however, the incidence of HCC continues to rise, attributed to the prevalence of alcoholic and obesity related liver diseases (3). Over the last two decades, several life prolonging advances have been introduced for the management of patients with early and intermediate stage HCC (4). Unfortunately, despite these advances and irrespective of etiology, surveillance strategies to detect early cancers are largely ineffective, resulting in late stage presentation for the vast majority. Options for these patients are limited and HCC incidence and annual mortality data remain remarkably similar.

There is an urgent need, therefore, to improve palliative treatment options for patients with advanced HCC. Cytotoxic therapies such as chemo or radiotherapy are poorly tolerated in patients with CLD and a major focus over the last few years has been on candidate targeted medical therapies. The multikinase inhibitor sorafenib is a cytostatic agent targeting RAF kinase and VEGFR signalling in the tumour cells and their microenvironment and following landmark trials published in 2008 and 2009, sorafenib became the standard of care for patients with advanced HCC (5,6). Although its survival benefit was a modest median of 6–10 weeks, its introduction was accompanied with enthusiasm and the hope that following this small but major step forward, second line therapies targeting alternative pathways would follow.

In fact, for a number of reasons as recently reviewed (7), this has not yet happened. Toxicity is partly to blame, but in addition has come the realisation that we need to understand better the key drivers of hepatocarcinogenesis, as well as how to block them effectively with emerging novel therapies. Biomarkers guiding treatment stratification may well be essential to guiding their use more effectively and we have entered a second phase of 'enrichment' trials in patients with HCC—treating individuals with upregulation of a targeted pathway, for example, rather than all comers. In addition to targeting oncogenic drivers more effectively, we have realised the need to improve our understanding of HCC therapy resistance and how to overcome it.

HCC has always been regarded as a notoriously treatment resistant cancer. Traditional cytotoxic therapies are not just poorly tolerated in cirrhotic patients—they are also largely ineffective. Recognised mechanisms of resistance include the upregulation of ABC transporters or pathways exporting or metabolising drugs in HCC cells. Strategies to target these pathways therapeutically have proved disappointing thus far. More recently has come the realisation that while an impairment of DNA damage repair can cause cancer, up-regulated DNA damage repair activity is often evident in established cancers (8). Both radiotherapy and cytotoxic drugs act by causing DNA damage, to which the cell mounts a DNA damage response (DDR) to signal and repair the damage. Increased DNA damage repair

activity can therefore contribute both to tumour survival and progression, as well as therapeutic resistance. For cancers whose survival is dependent on the DDR, there is hope that inhibition of the DDR may result in tumour death—with little damage to non-tumour tissues. In parallel is the hope that DDR inhibition may render traditional cytotoxic therapies more effective, at lower and better tolerated doses. Therapeutic targeting of DDR pathways may include treatments that inhibit DNA single-strand break (SSB) or double strand break (DSB) repair pathways. For example, base-excision repair of SSBs is dependent on the enzyme poly(ADP-ribose) polymerase (PARP). PARP inhibition is non-toxic and results in conversion of SSBs to DSBs. Trials suggest benefit in individuals who develop cancer as a result of a defect in DSB repair—namely those with germline BRCA1 or BRCA2 mutations (9). In these patients, cancer develops when a cell acquires a second mutation in the DSB DDR, but the cancer specific defect in DSB break repair becomes the cancer's 'achilles heel', as the cancer is consequently unable to repair the damage induced by PARP inhibition (10). In patients with HCC, germline BRCA1/2 mutations are rare, but PARP expression may be increased and have a role in HCC progression (11,12). Furthermore, PARP inhibition—possibly in combination with an agent promoting SSBs—may have therapeutic potential (13,14). Similarly, recent studies suggest that activity of the non-homologous end-joining pathway of DSB repair is upregulated in HCC, through increased expression and activity of the DNA-dependent protein kinase catalytic subunit (DNA-PKcs), and that this is a poor prognostic indicator contributing to the innate resistance of HCCs to cytotoxic agents (15,16). Inhibitors of DSB repair may therefore also have therapeutic potential in patients with HCC, if not as single agents, perhaps in combination with PARP inhibitors, or as potentiators of tumour directed cytotoxicity of lower dose chemotherapy or selective internal radiotherapy.

The paper by Nio *et al.* recently published in *Journal of Hepatology* (17), compliments the emerging theme of exploiting DDR inhibition for patients with HCC, but also sets this in the context of another proposed mechanism of resistance to cancer treatments—namely that of the so called 'cancer stem cell' (CSC). Within cancers it is proposed that a small minority of cells—CSCs—possess the characteristics of normal stem cells, retaining the ability to self-renew and differentiate into the multiple cell types present in a particular cancer. Of key importance is that CSCs often lack the particular characteristic targeted by a traditional or novel anti-cancer therapy. It is hypothesised therefore, that this small sub-population of cells are a distinct population that survive treatment and cause relapse as well as promoting metastatic disease. Strategically, therapeutic approaches specifically targeting CSCs may have the potential to treat cancers more effectively, reducing recurrence and metastatic spread.

Nio and the team lead by Taro Yamashita have previously shown that the stem cell marker EpCAM can be used to classify HCC subtypes with stem cell features, with distinct gene expression profiles and patient prognosis (18,19). They have also shown that cells sharing this phenotype exhibit resistance to chemotherapeutic agents (19,20) and have gone on to explore candidate underlying mechanisms. Using gene expression profiling approaches, they identified activation of the transcription factor Sal-like protein 4 (SALL4) in EpCAM positive HCC cells. SALL4 reportedly interacts with other stem cell transcription factors (e.g., Oct4 and Nanog), in addition to interacting directly with the epigenetic modulator and nucleosome remodelling and histone deacetylase (NuRD) complex—regulating histone modifications which maintain stemness. The NuRD complex is a chromatin remodelling complex, made up of chromodomain-helicase-DNA-binding proteins (CHDs), metastases-associated proteins and histone deacetylases (HDACs).

The authors have now highlighted the role played by chromodomain-helicase-DNA-binding protein 4 (CHD4)—a DNA-binding protein recruited to DNA damage sites in a PARP dependent manner—in the NuRD complex, exploring its contribution to chemoresistance in EpCAM positive HCC. Studying gene and protein expression profiles *in vivo* in 245 and 144 patients respectively, they have confirmed that CHD4 is abundantly expressed in EpCAM positive HCC in association with a poorer prognosis. Furthermore, they have manipulated CDH4 levels in EpCAM positive HuH7 HCC cells *in vitro*, showing that *CHD4* knockdown increased chemosensitivity to epirubicin, with reduced cell viability, while *CHD4* overexpression induced resistance, with increased cell viability in the presence of epirubicin. Having established a key functional role for CHD4, the authors have subsequently inhibited those functions of CHD4 that are mediated through HDAC and PARP, with specific respective inhibitors suberohydroxamic acid and AG-014699. Treatment with either agent reduced the numbers of EpCAM positive liver cancer cells *in vitro*, while having no impact on EpCAM negative HCC cell lines. Limited

inhibitor effects were observed *in vivo* in Huh7 EpCAM positive tumour xenograft growth in a mouse model, but the combination of HDAC and PARP inhibitor successfully inhibited xenograft growth, without any reported toxicity.

These data support an earlier study reporting synergy between inhibitors of chromatin modifying enzymes and PARP (21), but have taken a significant step forward in our mechanistic understanding of their effects and interaction. While SALL4 and the NuRD complex are clearly important in maintaining stem cells, these data suggest that in the presence of DNA damage in EpCAM positive HCC, SALL4 recruits CHD4 to the NuRD complex in a PARP dependent manner, promoting repair and chemotherapy resistance. Furthermore, inhibition of HDAC and PARP restores sensitivity to chemotherapy in EpCAM positive cells. These are promising data, presenting a therapeutic strategy to target chemoresistance in EpCAM positive HCC or EpCAM positive liver CSCs, potentially offering hope to a growing group of patients with a particularly poor prognosis. As stated by Nio *et al.* the safety, tolerability and efficacy of this or similar combinations for HCC patients warrants further investigation.

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Footnote

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