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Commentary

A novel function for HEG1 in promoting metastasis in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) remains one of the leading causes of cancer-related deaths around the globe. For patients receiving liver tumour resection, the risk of reoccurrence and metastasis is high. Cancer metastasis can occur as a consequence of a physical change known as epithelial to mesenchymal transition (EMT). In this instance, cancer cells acquire migratory and invasive characteristics that allow the cells to move into adjacent tissue or enter the bloodstream to reach a secondary site, where they begin to form a new tumour. Targetting proteins involved in the signalling pathways that induce the mesenchymal phenotype has been an ongoing field of research. A recently published study has described a novel role for the heart development protein with EGF-like domains (HEG1) in promoting EMT. This research provides new insights into the biological function of this protein in HCC. Furthermore, the research indicates a new target for future prognostic and therapeutic research in HCC.

The poor survival rate of hepatocellular carcinoma (HCC) is largely due to late-stage diagnoses and reoccurrence or metastasis of tumours. Although liver transplants are the most effective means of increasing survival rates, the limited availability of organ donors and the strict criteria for eligible HCC recipients results in most early-stage patients receiving resection [1,2]. Resection of HCC tumours have a high risk of reoccurrence and/or metastasis due to incomplete resection or *de novo* tumour formation [2]. Intrahepatic and extrahepatic metastasis can be attributed to de-differentiation of epithelial liver cancer cells into a mesenchymal phenotype. Known as the epithelial to mesenchymal transition (EMT), this process involves loss of epithelial markers, such as E-cadherin and the α- and γ-catenins, and increased expression of mesenchymal markers, such as N-cadherin and α-smooth muscle actin (αSMA), to promote local and microvascular invasion. Many pathways have been characterized in stimulating EMT, most notably is the potent action of transforming growth factor (TGF-β), which regulates the Wnt/β-catenin pathway to induce EMT and metastasis [3]. Activation of TGF-β/Wnt signalling is also associated with early HCC reoccurrence and poor patient survival [4]. Although these pathways have been identified in HCC patient subpopulations, finding reliable EMT biomarkers and therapeutic targets is still an ongoing field of research.

A recent published work by Zhao and colleagues [5] have described a novel function for the heart development protein with EGF-like domains (HEG1) in HCC. Although HEG1 is relatively understudied, its primary role is involved in cardiac development and endothelial cell junction formation [6,7]. Previous work has also implicated HEG1 as a highly specific marker for malignant mesothelioma [8]. In the more recent study, Zhao and colleagues [5] have elegantly described the function of HEG1 in HCC invasion and metastasis through the action of β-catenin to activate Wnt signalling and induce EMT.

The Wnt signalling pathway has been well described and normally serves important functions in embryonic development. Specifically, the Wnt/β-catenin pathway regulates the expression of genes involved in cell proliferation, cell survival and cell differentiation. In a series of events, Wnt ligands bind to a cell surface receptor, known as frizzled (FZD), which frees β-catenin from a repression complex [9]. β-catenin
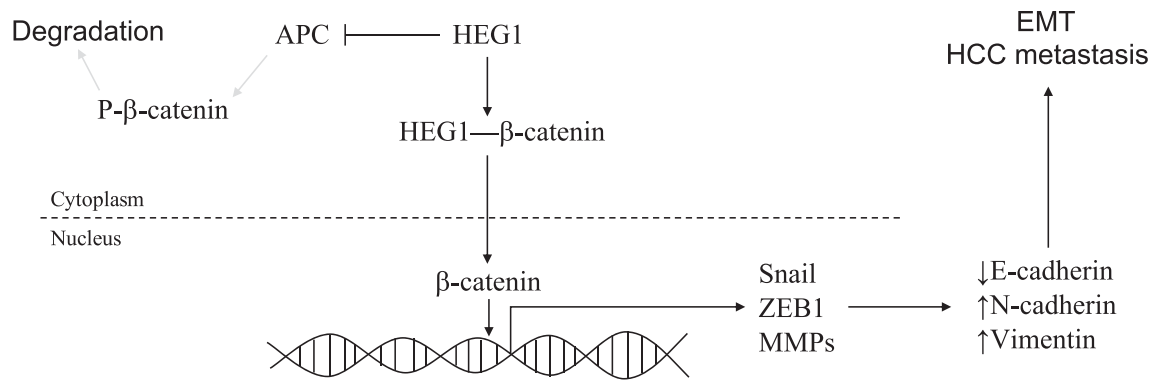
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Figure 1. HEG1 signalling in EMT

HEG1 stabilizes β-catenin and promotes localization to the nucleus. β-catenin induces expression of genes involved in activating EMT and promotes HCC metastasis. Abbreviations: APC, adenomatosis polyposis coli; HEG, heart development protein with EGF-like domain; MMP, matrix metallopeptidase; P, phosphate; ZEB1, zinc finger E-Box binding homeobox 1.

then accumulates in the cytoplasm and is translocated to the nucleus to act as a co-transcription factor for various genes involved in regulating cell behaviour [9]. Zhao and colleagues [5] have described a novel function for HEG1 as a protein that interacts with and stabilizes cytosolic β-catenin, while increasing translocation to the nucleus. Furthermore, the authors demonstrated that HEG1 may down-regulate the expression of adenomatosis polyposis coli (APC), which acts to repress β-catenin accumulation and target it for degradation (Figure 1) [5]. Previous studies have only implicated HEG1 as a transmembrane receptor protein [7,8]. However, the more recent study described HEG1 as localizing to the cytoplasm to interact with β-catenin [5]. The limiting number of studies regarding HEG1 function make it difficult to evaluate exactly how HEG1 localizes and functions within a cell, or within a cancer cell setting. However, previous work has suggested HEG1 to have similar structure and function to membrane-associated mucin proteins (MUC) [8]. Localization of MUC can occur in the membrane or the cytosol and is variable in malignant cancers, and high MUC expression has been associated with increased cancer metastasis [10]. It is probable that HEG1 functions similar to MUC in hepatic cancer, but more research is required to validate this highly specific role of HEG1 in cancer progression.

In addition, the authors of the present study have identified a close link with HEG1 expression and invasive mesenchymal HCC cell behaviour. They demonstrated that HCC cells overexpressing HEG1 had greater migratory capabilities *in vitro*. Furthermore, orthotopic xenograft models showed significantly greater incidences of intrahepatic and lung metastasis in tumour models with HEG1 overexpression [5]. This observation was further validated with functional analyses, demonstrating that HEG1 expression correlated with genes involved in EMT. More specifically, increased HEG1 expression correlated with loss of E-cadherin, which is an essential cell adhesion molecule required for maintaining normal epithelial cell interactions [5,10]. HEG1 expression also increased expression of the mesenchymal marker vimentin, which is in turn a significant component of the intermediate filaments required for mesenchymal cell integrity [11]. Low expression of E-cadherin and high expression of vimentin have previously been associated with a prognostic signature, along with high expression of mucin 1 (MUC1), for predicting poor prognosis in HCC [10]. Considering the structural and functional similarities between HEG1 and MUC1 [8], it may be concluded that a similar signature may be derived involving HEG1, E-cadherin and vimentin for predicting HCC patient outcome. As demonstrated by Zhao and colleagues [5], analyses of HEG1 expression in HCC patients further supports the prognostic value of this novel protein.

The present study used two patient cohorts totalling over 230 HCC samples to show a positive association with HEG1 expression and HCC prognosis. Patients with high HEG1 expression showed a distinct profile of larger tumours with vascular invasion and more advanced tumour node metastasis (TNM) and Barcelona Clinic Liver Cancer (BCLC) stages compared those with low HEG1 expression [5]. Furthermore, high HEG1 expression was significantly associated with decreased overall survival (OS) and disease-free survival (DFS) in both cohorts and was an independent risk factor for both shorter OS and DFS [5]. These data demonstrate compelling evidence of HEG1 as a specific marker of metastatic potential and poor prognosis in HCC. Typically, liver tumour specimens are examined for more classical prognostic factors such as BCLC or TNM tumour staging, or molecular factors including vascular invasion and α-fetoprotein levels. A combination of these factors often provides sufficient evidence to determine likelihood of

tumour reoccurrence. However, due to the complex nature of HCC, the tumour classification systems are not globally standardized and determining vascular invasion can be difficult to distinguish between the normal vasculature [12,13]. Measuring HEG1 expression quantitatively from a small piece of tissue or through histological analysis may be a simpler indication of metastatic activity. However, using HEG1 expression as a prognostic marker would also require standardizing a minimum expression level for poor prognosis indication. Nonetheless, the use of HEG1 as an addition to the current methods of evaluating patient prognosis may be valuable if validated further.

With this newfound biological understanding of HEG1 function in HCC cancer cells, Zhao and colleagues [5] suggest HEG1 may serve as a valuable therapeutic target. The authors demonstrated that HEG1 knockdown significantly reduced HCC cell invasion and metastasis *in vitro* and *in vivo*, by preventing EMT through up-regulation of APC [5]. While the authors show compelling evidence of the significant role of HEG1 in activating EMT, it is questionable whether HEG1 will be an effective therapeutic target. Previously noted, EMT signalling pathways are extensive, and there are many EMT triggers that may induce multiple pathways that could result in activating EMT. Other studies have investigated many relevant proteins that may be involved in such signalling pathways. For example, another study has shown that another membrane protein, metadherin (MTDH), has very similar effects on inducing EMT and metastasis in HCC [14]. This work also found a strong correlation with high MTDH expression and low E-cadherin expression, high vimentin and increased β-catenin localization to the nucleus. Furthermore, the present study reported significant correlational analysis with high MTDH expression and poor patient prognosis in HCC [14]. Both studies demonstrate mimicking results that indicate they can both independently activate Wnt signalling through β-catenin to activate EMT in HCC [5,14]. Whether or not HEG1 and MTDH function together to induce metastasis warrants further study. Nonetheless, this demonstrates only one example of the various factors that could contribute to metastasis in cancer progression. Targetting HEG1 as a therapeutic strategy to prevent metastatic HCC may only benefit a unique subset of patients who have high expression of HEG1. Although the efficacy of this is still contentious, considering it is likely that there are still many other active proteins within the heterogenic tumour capable of inducing EMT transition. Anti-HEG1 therapy may be more beneficial as a component of a patient specific anti-tumour drug cocktail delivered to target multiple relevant pathways catered to the patient in question.

The increasing incidence and poor survival rate of HCC remains a global issue, largely due to insufficient treatment options. Although liver transplants and tumour resection are curative options, the rate of reoccurrence is high and can be attributed to intrahepatic metastasis from primary tumour sites. The recent study by Zhao and colleagues [5] has demonstrated exceptional evidence for a novel role of HEG1 in activating EMT to induce metastatic activity in HCC. The clinical evidence describes a strong correlation with HEG1 expression and patient prognosis, suggesting it may be a valuable prognostic marker. Targetting the EMT pathway has yet to be a successful strategy for treating HCC patients, so it is unclear whether HEG1 would be an effective therapeutic target. Even so, the present study is the first to define a function for HEG1 in HCC development, and provides a new avenue for future research in defining EMT in cancer progression.

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Competing Interests
The authors declare that there are no competing interests associated with the manuscript.

Abbreviations
APC, adenomatosis polyposis coli; BCLC, Barcelona Clinic Liver Cancer; DFS, disease-free survival; EMT, epithelial to mesenchymal transition; FZD, frizzled; HCC, hepatocellular carcinoma; HEG1, heart development protein with EGF-like domain; MTDH, metadherin; MUC, mucin protein; OS, overall survival; SMA, smooth muscle actin; TGF-β, transforming growth factor; TNM, tumour node metastasis.

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