Reviewer #2:

1 Tables and figures should be simplified and made concise and easier to understand for the readership of the journal.

Response:

The original Figure 4 was reorganized to be new Figure 4 and Supplementary Figure 6. The original Table 3 was relocated to supplementary data to be Supplementary Table 6. The original supplementary figure 4 and 5 were combined to be Supplementary Figure 5. The original supplementary figure 6 and 7 were combined to be Supplementary Figure 7. The original supplementary figure 8 and 9 were combined to be Supplementary Figure 8.

2 PGRMC1 has the biochemical function of heme-binding. It is necessary to discuss this function and HCC status.

Response:

PGRMC1 contains a motif common to heme-binding proteins, suggesting a role in oxidative metabolism[1]. Free heme, i.e. heme not appropriately bound by hemoproteins or heme-binding proteins, is a powerful pro-oxidant agent and therefore potentially toxic[2]. Heme binding proteins are required to maintain cellular stasis and to detoxify cells. Excessive heme iron has been reported to increase the risk for several types of cancer, such as colon cancer,[3] gastric cancer[4], esophageal cancer[5], and HCC[6]. As a heme-binding protein, PGRMC1 has been reported to interact with P450 proteins and protect cells from DNA damage [7-9]. Therefore, PGRMC1 may protect hepatocytes from oxidative stress and suppress carcinogenesis by appropriate heme delivery or heme containment. The explanation was added in the discussion section (Page 20).

3 PGRMC1 is widely expressed in the human body. The significance of its expression in HCC tissue and normal tissue of the same patient is limited. It is necessary to investigate the difference in its expression between the HCC patient and the health patient.

Response:

Expression of PGRMC1 in health liver was shown in Supplementary figure 1.

Expression levels of PGRMC1 among normal liver of healthy individuals, non-cirrhotic and cirrhotic liver of HCC patients were provided in supplementary figure 4A. We found no significant difference between liver of HCC patients and that of healthy controls. The differences of PGRMC1 expression between non-tumor liver and HCC in the same patient, irrespective of cirrhosis, were also provided in supplementary figure 4 C and D. Expression of PGRMC1 was downregulated in HCC tissue compared to non-tumor liver in both groups of patients. PGRMC1 was not significantly altered in progression from normal healthy liver, non-cirrhotic hepatitis liver to cirrhosis. Since PGRMC1 was significantly downregulated at HCC stage, PGRMC1 is thought to play a role in the HCC progression. The interpretation was added in P15-16.

4 According to existing studies, PGRMC1 is overexpressed in breast cancer and other malignancies, which is different from the results of this paper and needs explanation.

Response:

Sex hormones could exert different tumorigenic properties depending on the tissue type. For example, contrary to their hypothetical protective role in liver cancer development, chronic exposure to estrogens favors carcinogenesis in the breast and uterus. PGRMC1 may have different expression statuses and functional roles in different cancers. Expression of PGRMC1 was shown to be upregulated in breast cancer and ovarian cancer and was found to be associated with advanced stage or poor prognosis.[10, 11] Its expression was more often detected in ER-negative breast cancers[10], and it may act via cross-talk with nuclear or extranuclear ER receptors.[12] PGRMC1 has been localized in hypoxic areas of breast cancer[10] and demonstrated to activate expression of vascular endothelial growth factor in glial cells.[13] A D120G mutant of PGRMC1 increases the susceptibility of breast cancer cells to doxorubicin and camptothecin treatment.[14] However, PGRMC1 has been reported to be associated with EGFR in lung cancer cells and to enhance susceptibility to the EGFR inhibitor, erlotinib. [15] Overexpression of PGRMC1 in the MCF-7 breast cancer cell line sensitizes cancer cells to hydrogen peroxide treatment with corresponding hyperphosphorylation of Akt and IkB proteins.[7] These findings suggest that PGRMC1 plays a plethora of biological roles in human cancers. In contrast to breast and ovarian cancer, PGRMC1 is downregulated in HCC. PGRMC1 is located at

chromosome Xq22-q24. A prior genomic study found a frequent LOH of Xq (43%) in HCC [16] with a progressive increase in fractional allelic imbalance from cirrhotic nodules at progressive stages (11% - 57%) to HCC, suggesting its involvement in the hepatocarcinogenesis. Furthermore, let-7/miR-98 was reported to repress PGRMC1[17, 18], and gradually elevated miR-98 has been associated with the progression of liver cancer.[19] Therefore, microRNA could be an alternative regulatory mechanism in suppression of PGRMC1 expression. Further study is needed to clarify the mechanisms of PGRMC downregulation in HCC. The discussion was added in Page 22.

5 Typo and grammatical errors exist.

Response:

The typo and grammatical errors have been corrected as requested. The manuscript has been English edited.

Reviewer #3:

This is very interesting paper about the relationship between HCC and progesterone. It is noted that the HCC risk was inversely related to the age at natural menopause. Oophorectomy performed at age 50 or younger during premenopausal years was also a risk factor for HCC, suggesting that at least female sex hormones including progesterone or estrogen may be protective against HCC.

1.According to auther's paper (Suppelmetary Fig 2), progesterone receptor is much higher in HCC tumor than non tumor, but estrogen receptor is in HCC than non tumor. Please tell me the reason why progesterone receptor is much higher in HCC tumor than non tumor,. Interestingly, cirrhotic patients with HCC have significantly lower plasma concentrations of testosterone, dihydrotestosterone, and dehydroepiandrosterone than patients with cirrhosis alone. Low levels of testosterone in male HCC patients and high levels of progesterone in cirrhosis patients have been observed. It is controversial that high levels of progesterone are associated with premalignant cirrhosis. Do the higher progesterone levels contribute to HCC development?

Response:

Previous studies have shown that high levels of progesterone can be observed in patients with cirrhosis[20]. This is likely due to impairment of progesterone metabolism in the liver. It is controversial whether high levels of progesterone are associated with premalignant cirrhosis. Previous studies have shown that the occurrence of natural menopause at a younger age or oophorectomy performed at age 50 or younger is associated with increased risk of HCC [21, 22]. PR expression in HCC has been correlated with better prognoses[23]. These findings suggest that progesterone may be protective against HCC. Furthermore, progesterone can serve as the precursor for the major steroid hormones (androgens, estrogens, and corticosteroids). The oncogenic effects of androgen and the protective effects of estrogen and progesterone in liver may also depend on the hormonal receptors expressed on hepatocytes or cancer cells[21]. The discussion was added in Page 21.

2.Please comment about the higher progesterone levels contribute to HCC development.

Response:

Previous study reported that younger age of natural menopause or oophorectomy performed at age 50 or younger is associated with increased HCC risk[21, 22]. PR expression in HCC correlated with a better prognosis[23]. These findings suggest that progesterone may be protective against, rather than contribute to, HCC development. The discussion was added in Page 21.

3.Please tell me the reason why PGRMC1 is prognostic factor, but PGRMC2 is not prognostic factor.

Response:

PGRMC1 differs from PGRMC2 in the transmembrane domain and N-terminals, resulting in diverse interaction partners in the lumen of sub-cellular organelles or on the cell membrane surface. The SH3 target sequence of PGRMC1 and its consensus CK2 site is also absent in PGRMC2, implying that PGRMC2 may not interact with SH3-containing proteins[24]. Therefore, these two proteins may have different

interacting partners in connecting with cellular membrane, organelles and cell signaling molecules. Furthermore, PGRMC1 has also been proposed as a sigma-2 receptor [25] with capability to inhibit tumor growth [26, 27]. In our study, PGRMC1 was inversely associated with AFP, tumor differentiation and AJCC stage compared with PGRMC2. These may be the reasons why PGRMC1 downregulation is more significantly associated with tumor progression and worse prognosis. In multivariate analysis, PGRMC1 was an independent parameter in predicting patients' survival in two different cohorts. Therefore, only PGRMC1 was proved to be a prognostic biomarker in HCC. The discussion is added in Page 19.

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