Sex hormone-binding globulin impedes hepatocellular carcinoma growth via MERTK regulation

Sex hormone-binding globulin (SHBG), a crucial modulator of sex steroids in human blood, regulates androgen bioavailability and homeostasis.1 When androgen or AR signaling is known to be involved in the hepatocellular carcinoma (HCC) and SHBG plays an integral role in regulating the levels of bioavailable androgens, the concern whether SHBG might influence HCC onset or progression by modulating androgen action remains open. Our study aims to investigate the role of SHBG in HCC progression in contexts of androgen regulation and steroid independence.

In clinical data, SHBG mRNA expression in tumors was significantly lower than adjacent non-tumor liver tissues (Fig. 1A, S1A), and was significantly lower in HCC patients compared to non-HCC patients (Fig. S1B). Interestingly, long tumor doubling time and small tumor size were observed in HCC patients possessing high SHBG expression (Fig. S1C,D). Immunofluorescent SHBG protein level was markedly suppressed in HCC grade 4 (Fig. S1E). Furthermore, overall survival duration was significantly extended in HCC patients with high SHBG expression (Fig. S1F,G). It also should be noted that patients expressing tumor SHBG levels similar to those of NT have a better prognosis. To investigate how SHBG affects HCC, we induced HCC in 2-week-old male mice expressing human full-length SHBG gene (SHBG-Tg) and evaluated the tumor progression at 40-week-old. Representative gross images and H&E staining of tumors were associated with reduced the number of tumor nodules, as evidenced by the sum and average in size of tumor nodules (Fig. 1C−E, S2A). Consistent with clinical data, SHBG mRNA level in the tumor was lower than that in the normal liver parenchyma (Fig. S2B), but SHBG protein was significantly concentrated in the HCC region (Fig. S2C, D). Likewise, acute diethylnitrosamine (200 mg/kg) administration to SHBG-mice or SHBG-administered mice showed a similar SHBG expression tendency (Fig. S3, 4). Since SHBG internalization and SHBG receptor are not clear, we hypothesized that extracellular-accumulated SHBG exists and would play a role in HCC regulation.

The plasma of SHBG-Tg mice significantly decreased HMGB1 level, which represents necrotic DAMPs (Fig. 1F). Conversely, tumors from SHBG-Tg mice showed significantly higher apoptotic gene levels and TUNEL positive signals, and had lower PCNA levels and proliferation-related transcripts (Fig. 1G−K). As apoptosis minimizes inflammation while necrosis produces inflammatory content, proliferation rates of SHBG-tumor should be affected by inflammation. Indeed, IL-1β levels and pro-inflammatory mRNA levels were suppressed in tumor-bearing SHBG-Tg mice (Fig. 1L, S5A). Furthermore, SHBG-Tg mice decreased levels of fibrotic markers, invasiveness markers, and C-MET signaling proteins which are related to inflammation (Fig. S5B−D). Through presented results, we inferred that SHBG suppresses DAMPs production from necrotic hepatocytes and hinders pro-inflammatory response from Kupffer cells. Though SHBG regulates androgen bioavailability, pro-inflammatory cytokines were not markedly affected by testosterone in Raw 264.7 cells (Fig. 56). However, Raw 264.7 cell activation with high IL-1β were substantially suppressed in SHBG plasma or pure SHBG-treated group (Fig. 1M, N, S7), suggesting SHBG could play as a signal-modulator in HCC progression regardless of testosterone engagement.

Along with increased HCC risk in androgenic females,2 testosterone withdrawal in WT and SHBG-Tg mice decreased HCC progression (Fig. S8A). When plasma testosterone levels were substantially higher in SHBG-Tg mice than in WT mice at a 40-week-old (Fig. 1O), the AR level and AR target gene levels were suppressed in SHBG-tumor (Fig. 1P, S8B). Like mouse model, SHBG...
overexpression reduced nuclear AR and AR target gene levels in SNU423 cells (Fig. S8C, D). Accordingly, plasma SHBG is shown to suppress androgenic action following the free-hormone hypothesis. This could influence the cancer cell survival because testosterone increases HCC cell growth in AR overexpressed SNU423 cells (Fig. S9). Indeed, SHBG-overexpressed cells significantly suppressed the cell growth in testosterone treatments (Fig. S8E) and increased apoptotic PARP regardless of androgen regulation (Fig. S8F, G).
Hence, the following study focused on that SHBG had a steroid-independent role in the suppression of cancer cell proliferation. SHBG-overexpressed SNU423 cells decreased proliferation-related transcripts and cell proliferation rates without steroid hormones (Fig. 1Q, R). In DAMPs incubation, SHBG-overexpressed cells increased apoptotic PARP and suppressed proliferation rates (Fig. 15, S10). These results imply that SHBG independently restrains cell proliferation in HCC beyond the canonical suppressive role of testosterone modulator. Substantially, SHBG-tumors, SHBG-overexpressed SNU423 cells, and SHBG-treated Raw 264.7 cells suppressed MERTK phosphorylation, which relates to suppression of cancer cell death and promotion of cancer cell proliferation. These might be related to the extracellular accumulation of SHBG, which can hinder the DAMPs-mediated MERTK activation since the SHBG domain of Gas 6 binds to MERTK. MERTK phosphorylates mitogen-activated protein kinases (MAPK) which are responsible for C-Jun and C-Fos expression and the nucleus translocation of AP-1, promoting the proliferation of cancer cells. Under the SHBG condition, MAPKs activation and AP-1 levels were substantially decreased following low MERTK phosphorylation, suggesting that SHBG is a crucial regulator for HCC progression via the MERTK-AP-1 axis (Fig. 1T, S11,12).

Collectively, the present study reveals the SHBG’s role during HCC progression in the context of steroid-dependent and steroid-independent regulation. As a crucial modulator for androgens, SHBG suppresses androgen-mediated cancer cell proliferation in AR dominance. Beyond its role as a steroid regulatory protein, SHBG independently increases apoptosis and decreases proliferation of cancer cells, and suppresses pro-inflammatory IL-1b production of macrophage. MERTK phosphorylation and JNK/p38/ERK signals were substantially suppressed by SHBG, thereby lowering activation of AP-1 complex which is closely associated with cancer cell proliferation and IL-1b production of immune cells. Through our findings of SHBG physiology, as a clinical marker and therapeutic strategy for HCC, SHBG should be highlighted for suppressing cancer cell proliferation and pro-inflammatory response in the HCC environment.

Author contributions

SRL and EJH designed research; SRL and EJH performed research; SRL and EJH analyzed data; SRL and EJH wrote the paper.

Conflict of interests

Authors declare no conflict of interests.

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Appendix A. Supplementary data

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