The connection of β -catenin and phenobarbital in murine hepatocarcinogenesis: a critical discussion of Awuah et al., PLoS ONE 7(6):e39771, 2012

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Phenobarbital (PB) is frequently used as a promoter of rodent liver tumors. The mechanisms by which PB exerts its tumor-promoting activity are still not fully understood, but the constitutive androstane receptor (CAR) and β -catenin seem to be essentially involved: Experiments with *Car* knockout (KO) mice have proven that the presence of CAR is mandatory for PB-mediated tumor promotion (Yamamoto et al. 2004). In mice, PB selects for the outgrowth of hepatocellular tumors with activating mutations in the *Ctnnb1* gene, which encodes the transcription factor β -catenin (Aydinlik et al. 2001). Similar observations were made with the PB-like tumor promoter PCB153 (Strathmann et al. 2006). Furthermore, CAR and β -catenin interact in the regulation of enzyme induction and hepatocyte proliferation triggered by PB (Braeuning et al. 2009; Braeuning et al. 2011).

In the absence of PB, two groups have studied hepatocarcinogenesis induced by diethylnitrosamine (DEN), a potent tumor initiator, in β -catenin-deficient livers from transgenic mice with conditional hepatocyte-specific KO of *Ctnnb1*. The unexpected finding of both studies was an increased number of tumors in *Ctnnb1* KO mice, which contradicts the classic view of β -catenin as an oncogenic driver of tumor formation (Rignall et al. 2011; Zhang et al. 2010). Possible explanations for this finding are increased inflammation and oxidative stress in the KO mice (Zhang et al. 2010), as well as the increased number of hepatocytes with high levels of O⁶-ethylguanine after DEN injection (Rignall et al. 2011). Subsequent administration of PB promoted tumor formation in livers of wild-type (WT)

C3H mice, whereas a lack of tumor promotion was observed in *Ctnnb1* KO mice in the same experiment (Rignall et al. 2011). This can be attributed to the fact that the target cells of PB-mediated tumor promotion, namely *Ctnnb1*-mutated hepatoma cells, are largely absent from *Ctnnb1* KO livers.

The group of Dr. Monga has recently reported an increase in liver tumor burden of DEN/PB-treated *Ctnnb1* KO mice, as compared to WT controls (Awuah et al. 2012). This seemingly contradicts previous findings (Rignall et al. 2011) and thus questions the established connection of PB and β -catenin, at least for readers who are not highly specialized in the field of tumor promotion.

In their recent study, the authors treated 2-week-old C57BL/6 mice with a single dose of DEN, followed by chronic exposure to PB (Awuah et al. 2012). The age of mice at DEN injection is highly relevant since, in C3H mice, it has been shown that PB inhibits hepatocarcinogenesis when the initiator DEN is given to 2-week-old mice, whereas it promotes tumorigenesis after DEN injection at 6 weeks of age (Moennikes et al. 2000). Comparably, PB inhibits tumor development in DEN-induced (2 weeks) mice from strain B6C3F1, a crossbreed of C3H and C57BL/6 (Diwan et al. 1984; Klaunig et al. 1987, 1988; Lee et al. 1998). The paradoxical phenomenon of tumor inhibition by PB has been reviewed by Lee (2000). Using CD1 mice, however, Tamano et al. (1994) have demonstrated tumor promotion by PB in animals treated with DEN at 2 weeks of age. Therefore, it cannot be excluded that PB has acted as a tumor promoter in the study by Awuah and colleagues. However, the majority of available studies argue against a tumor-promoting activity of PB when using the 2-week DEN treatment regimen, especially since the C57BL/6 strain is rather refractory to liver tumor formation and promotion (Maronpot 2009).

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Unfortunately, the study by Awuah et al. (2012) lacks the DEN-only control groups which could clarify whether tumor promotion has occurred in their study or not. Therefore, presented data do not allow the conclusion that PB has an impact on tumor development in *Ctnnb1* KO mice. Supposed that PB was inactive as a tumor promoter in their experiment, the result that *Ctnnb1* KO mice developed more tumors than their WT counterparts would be in accordance with previous results on non-PB-treated mice (Zhang et al. 2010; Rignall et al. 2011).

Indirect evidence for the above assumption that PB did not act as a tumor promoter in their study comes from the author's notion that the "utilized DEN/PB protocol in the WT C57BL/6 mice did not select for β -catenin gene mutations during hepatocarcinogenesis" (Awuah et al. 2012): PB and similarly acting compounds selectively promote the outgrowth of tumor cells with activated β -catenin in C3H (Aydinlik et al. 2001), B6129SF2/J (Strathmann et al. 2006), and C57BL/6 mice (Marx-Stoelting et al. 2009; own unpublished analyses of tumors from the latter experiment). In addition, in *Ctnnb1* KO livers, chronic PB treatment provides a selective advantage to the few remnant β -catenin-possessing hepatocytes, as compared to surrounding *Ctnnb1* KO cells (Braeuning et al. 2010). It has also been shown that PB stimulates the growth of eosinophilic lesions while inhibiting the outgrowth of basophilic lesions in mouse liver (Lee 2000; Lee et al. 1998). Figure 1C of the paper by Awuah et al. (2012) shows a selection of liver lesions from their experiment which appear to be rather basophilic in nature.

In summary, the design of the latter study, lacking DEN-only control groups, makes it impossible to decide whether or not PB has acted as a tumor promoter in the study by Monga's group. In view of the published literature cited above, it seems likely that PB had no promoting effect on tumorigenesis in the study by Awuah and colleagues. Therefore, the study does not necessarily conflict with previous results demonstrating an absence of PB-mediated tumor promotion in *Ctnnb1* KO mice (Rignall et al. 2011).

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