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Species differences might lead to misleading conclusions regarding human cancers. We agree that the mechanism of glucocorticoid production is important, and, therefore, we analyzed CYP11B1 (synthesis) and HSD11B1 (regeneration) expression in human cancers. HSD11B1 but not CYP11B1 was upregulated in multiple cancers and was widely and strongly associated with expression of glucocorticoid-responsive genes, regulatory T cell markers, and effector T cell exhaustion markers. This paralleled our findings in mice and strongly supports the notion that 11β-HSD1 (not Cyp11b1) produces biologically significant levels of cortisol in these human tumors, with a negative clinical impact. Our findings have been mirrored by those of another group independently converging on 11β-HSD1 as a target for cancer therapy, using mouse models and human cancer data sets (5).

De novo-synthesized corticosterone might have been produced but then converted to aldosterone by Cyp11b2. Cirillo supposes that

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**Figure 1. Glucocorticoid-producing enzyme expression in tumors.** (A) Relative Hsd11b1, Cyp11b1, and Cyp11b2 expression (corrected for Gapdh) in tumor cells was determined by reverse-transcription qPCR. (B) 2.5 × 10⁴ tumor cells were cultured with 100 nM DOC or DHC for 24 hours, and steroids were assayed by ELISAs (Arbor Assays). (C) Relative gene expression in human cancers. TCGA gene expression data were analyzed using Gene Expression Profiling Interactive Analysis (http://gepia.cancer-pku.cn/), and median values are shown.
the tumor cells we analyzed did synthesize corticosterone via Cyp11b1, but that it was all converted to aldosterone by coexpressed Cyp11b2. If this were true, the synthesized corticosterone would simply be an intermediary and transient metabolite, not a secreted bioactive product. More importantly, 11β-HSD1–generated corticosterone from the same cells would also have been depleted by conversion to aldosterone. This point exposes a self-contradiction in this model: efficient corticosterone conversion to aldosterone would preclude its detection from all sources. Nonetheless, we have tested this possibility and found that Cyp11b2 expression was extremely low in B16.F10 (B16) melanoma and MC38 colorectal tumor cells (Figure 1A). Although both converted DHC to corticosterone, neither converted DOC to corticosterone or aldosterone (Figure 1B), ruling out a role of Cyp11b2.

Our study does not contradict previous evidence of tumor synthesis of glucocorticoids. We did not claim that tumors can never synthesize biologically significant levels of glucocorticoids, and some certainly do (this clearly occurs with adrenal adenomas and, perhaps, murine AOM-DSS–induced colorectal tumors, ref. 6). However, for the seven glucocorticoid-producing tumor cells we examined, the amounts regenerated by 11β-HSD1 were vastly greater than those synthesized by Cyp11b1, and inhibiting 11β-HSD1 substantially enhanced antitumor immunity and reduced tumor growth. In humans, HSD11B1 was expressed in many cancer types, but CYP11B1 and CYP11B2 had low or absent expression (Figure 1C). The data thus far support the notion that 11β-HSD1–mediated regeneration, not Cyp11b1–mediated synthesis, is the major source of biologically, and perhaps clinically, relevant tumor-derived glucocorticoids.

Data availability. Values for all data points in graphs are reported in the Supporting Data Values file.