tended to inhibit proliferation of allogeneic $CD4^+$ T cells (data not shown). Using this culture, we demonstrated that DCs are indispensible for IL-10^+ $CD25^-FOXP3^-$ Tregs induction *in vitro* by way of PD-1/PD-L1 and IL-T4/HLA-G pathways. Several reports showed that such molecular interactions are involved in the generation of regulatory cells in cancer patients.[29,30] In patients with HCC, a positive correlation is observed between the expression of PD-L1 or HLA-G in cancer tissue and the poorer prognosis of the patients,[31,32] suggesting that such molecules are involved in cancer development. As for HLA-G in this study, direct cellular contact between DC and HCC is not necessary in IL-10^+ $CD25^-FOXP3^-$ Tregs induction, suggesting that soluble HLA-G released from HCC may play an active role. In our hands, soluble HLA-G was measurable in culture supernatants of HCC cell lines and in serum samples from HCC patients (data not shown). Further investigation is arguably needed to elucidate whether soluble HLA-G is functional or not in HCC patients.

In summary, we demonstrate that $CD25^-FOXP3^-$ Tregs are increased in HCC patients, which change dynamically in response to HCC occurrence and post-therapeutic recurrence. Cross-talks among HCC cells, DC and $CD4^+$ T cells are required for IL-10^+ $CD25^-FOXP3^-$ Tregs induction, in which PD-L1, HLA-G and IL-T4 are critically involved. Although further investigation is needed to prove that deprivation or inactivation of $CD25^-FOXP3^-$ Tregs improves immune responses *in vivo*, such molecules could serve as targets of Treg-oriented therapeutic intervention for HCC.

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