Response

We appreciate the interest of Dr. Cristobal and his colleagues in our study. The main objective of our study was to elucidate the role of oncogenic *K-Ras* mutation in cancer stem cell (CSC) activation contributing to colorectal cancer (CRC) tumorigenesis. We showed that oncogenic K-Ras enhances tumor progression and metastasis by activation of CSCs in CRC harboring *APC* mutation. The highlight of the crosstalk between Wnt/β-catenin and Ras/ERK signaling pathways is the stabilization of mutant K-Ras protein by *APC* loss, which triggers further activation of the Wnt/β-catenin signaling for CSC activation in CRC cells (1). These findings suggest that targeting both hyperactive Wnt/β-catenin and the Ras/ERK pathways may be an effective combination therapy for the treatment of CRC, because *APC* (90%) and *K-Ras* (30–40%) mutations are among the most common abnormalities occurring in human CRC.

Dr. Cristobal et al. suggested investigating an alternative molecular mechanism regulating the β-catenin pathway that could be involved in modulating CSC activation in order to further determine the role of K-Ras as a key regulator of CSC activation. They considered Caveolin-1 (Cav-1) as a modulator of CSC activation. Cav-1 has been reported as a target of K-Ras, functioning as a positive feedback regulator of K-Ras signaling in CRC (2) and is also involved in the regulation of the Wnt/β-catenin pathway (3–5). However, according to their observations, the Cav-1 overexpression effects on the decrement of β-catenin levels, reduction in colonosphere formation ability, and downregulation of CSC markers (CD44, CD133, and CD166), were only shown in HT-29 cells harboring wild-type *K-Ras*. These effects were not observed in *K-Ras* mutated DLD-1 or SW480 cells. The loss of the inhibitory role of β-catenin signaling by Cav-1 in *K-Ras*–mutated CRC cells may further enhance β-catenin signaling and CSC activation. It could be an alternative mechanism for CSC activation by oncogenic *K-Ras* mutations in CRC, consistent with our finding that oncogenic *K-Ras* mutations play a role in CSC activation, although we provided a different mechanism involving initial activation of β-catenin by *APC* loss and subsequent enhanced β-catenin signaling via stabilization of oncogenic K-Ras. It is plausible that oncogenic *K-Ras* mutations may activate β-catenin signaling and CSCs by inhibiting the function of Cav-1, possibly via unrevealed mechanisms, which could be attractive subjects for future studies.

Although the role of Cav-1 in modulating the Wnt/β-catenin pathway is limited to the wild-type *K-Ras* HT-29 cells, these observations are of interest in the cancer research community because of the extensive involvement of Cav-1 in cancer. The role of Cav-1 in cancer development has been a subject of controversy partly owing to the complex heterogeneity of the genetic background of CRC cells. Therefore, in order to clarify the role of Cav-1 in CSC activation in CRC, adopting *APC*-mutated isogenic CRC cells harboring either wild-type or mutant *K-Ras* is recommended. Moreover, investigating the role of Cav-1, which is supposed to be upregulated in the presence of oncogenic *K-Ras* mutations, should aid in understanding its role in CRC.

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