Author's responses to Rewiewer #1
Reviewer's code: 03303427

Scientific Quality: Grade C (Good)
Language Quality: Grade B (Minor language polishing)
Conclusion: Major revision

Authors would like to thank the reviewer for his thoughtful responses, which we feel have improved the manuscript. Below are all of his suggestions and concerns followed by our responses

1. In this manuscript, the authors did not describe if there's inclusion criteria of CRC patients.

   In order to obtain a sample reflecting the general population, the study was designed with wide range of inclusion criteria. Patients were excluded from the study if they met the following criteria: 1) age <35 years, 2) have known genetic predisposition to the development of colon cancer. We analyzed material from patients who underwent radical surgery with lymph node dissection. Small biopsy specimens were excluded from the study.

2. Could you please explain the underlying mechanism that there are positive correlations between IRS-1 and both Bax and Bcl-xL.

   Our study suggests that IRS-1 is co-expressed with both pro- and antiapoptotic markers and all these proteins are more prevalent in more differentiated CRC than in poorly differentiated CRC. It could have several possible interpretations. Coexpression of IRS-1 and Bcl-xL support the thesis that IRS-1 promotes cells viability and enables abnormal proliferation of tumor cells. In general, cell survival depends on the balance between expressed amounts of proapoptotic and antiapoptotic Bcl-2 family members but function of Bcl-2 family proteins could be also regulated by its phosphorylation or dephosphorylation rates. It was reported that IRS-1 suppress apoptotic cell death induced by growth factor withdrawal probably through regulating phosphorylation of some proteins from the Bcl-2 protein family, including Bcl-xL (but not Bax protein). It was also reported that overexpression of the IGF-IR in human CRC cell line results in up-regulation of the Bcl-xL. It seem reasonable to consider that Bax and Bcl-xL expression can be independently regulated by the signaling pathway such as IGF-IR/IRS-1 and by some microenvironmental conditions, *e.g.* oxidative stress in case of more advanced tumor with high cellular density. Another study

demonstrated that IGF-1 treatment of osteosarcoma cells stimulated growth and proliferation but also mildly induced apoptosis. Thus, it can be concluded that the increased growth induced by IGF-1 treatment is balanced by activation of pro-death mechanisms, which might provide balanced cell turnover and result in tumor progression.

3. Maybe this study is not profund enough. It will be better if there are some researches about mechanism and deeper relationships among IRS-1 and apoptotic markers.

   The current study adds to a growing corpus of research showing that 1) IRS-1 expression is more prevalent in more differentiated tumors, and our data indicate that 2) IRS-1 expression is correlated with both proapoptotic Bax and antiapoptotic Bcl-xL proteins, but the authors agree that this results are not conclusive enough to explain the precise mechanisms of how IRS-1 interplays with apoptotic proteins. Our data show only coincidence of IRS-1 and apoptotic proteins expression. Therefore, future investigations are necessary to validate the kinds of conclusions that can be drawn from this study.