Author's response to reviews

Title: Clinical significance of OCT4 and SOX2 protein expression in cervical cancer

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Author's response to reviews: see over
We would like to thank the reviewers' constructive comments, which have allowed us to significantly improve the manuscript. We have provided a point-by-point description of the changes below and have highlighted them in red in the revised version of the manuscript.

**Response to Reviewer 1**

Kim and collaborators investigated the clinical significance of OCT4 and SOX2 transcription factors in cervical cancer. Protein expression was evaluated by IHC (employed in a semi-quantitative mode) in 161 primary invasive cervical cancers, 289 cervical intraepithelial neoplasias and 305 matched normal tissue samples. IHC results were related to clinicopathological features, including survival rates of patients; survival analysis proved that OCT4 overexpression and SOX2 loss of expression were associated with poor prognosis in cancer patients. The question posed by the authors is well defined; methodological approaches, number of investigated cases, and statistical evaluation are perfectly adequate to meet the purpose of the work. The result obtained are clearly presented, properly interpreted, and discussed in the frame of relevant literature. Accordingly, I have no criticisms about this paper.

We thank the reviewer for these positive comments.

**Response to Reviewer 2**

The author must respond to these before a decision on publication can be reached. Kim et al, presented their work entitled Clinical significance of OCT4 and SOX2 protein expression in cervical cancer. They have suggested that OCT4 over expression and loss of SOX2 expression are strongly associated with poor prognosis in patients with cervical cancer. But recently published papers (Jing Ji et al., 2014, Chang et al., 2015, and Wen Li et al.,) with same type of work need to be considered, before final judgment:

1. The author should validate and justify their findings with the recently published papers as mentioned below:

We appreciate the reviewers' constructive comments. We reviewed 3 papers and have revised in the discussion section.

a) Jing Ji et al., Int J Clin Exp Pathol 2014;7(5):2470-2476, Reported that Sox2, and Oct4 were highly increased in CSCC compared with the normal cervix tissues.
Ji et al. reported SOX2 and OCT4 were highly increased in cervical cancer compared with normal cervix. This finding is in agreement with our result and we added this finding in the discussion section as follows:

[On page 10-11, Discussion, Line 217-226]

OCT4 and SOX2 are important transcriptional factors involved in maintenance of pluripotency and self-renewal in cancer stem cells, aberrant expression of OCT4 and SOX2 might contribute to carcinogenesis in various cancers [15, 21, 22]. Radioresistance is important in the treatment and prognosis of cervical cancer and it is known to be associated with cancer stem cells [3]. This study examined the clinical correlation and prognostic significance of stemness-related OCT4 and SOX2 protein expression assessed by IHC in premalignant and malignant cervical tumors. The results demonstrate that OCT4 and SOX2 protein expression is elevated in premalignant and malignant cervical tumors compared to normal cervix and this finding is consistent with a previous study [23]. OCT4 was an independent poor survival factor but SOX2 showed as a favorable prognostic factor.

b) Chang et al., Tumor Biol (2015) reported that 100 % of well-differentiated and 66.7 % of moderately differentiated cervical SCCs showed lower SOX-2 expression, while 58.8 % of poorly differentiated tumors had higher SOX-2 expression

We have revised and added references in the discussion section as follows:

[On page 12, Discussion, Line 250-269]

SOX2 is known to play an important role in regulating the cell cycle, DNA repair, and self-renewal in stem cells [30]. It is associated with tumorigenesis, chemoresistance and maintenance of stem cell-like property in cancer cells, which suggests poor overall survival [16, 31, 32]. High expression of SOX2 was reported to be associated with a lack of cell differentiation and to contribute cell migration and invasion in cervical cancer cell line [33]. In addition, Shen et al. showed that SOX2 is highly expressed in patients with radiation resistance and predicts poor survival [29]. In contrast, SOX2 expression was associated with prolonged survival in the current study. These discrepancies might be explained by the lack of standardized methodology, different standards of interpretation, or differences in studies’ patient populations. Similar to our study, Wilbertz et al. reported that SOX2 gene amplification and protein expression are associated with favorable survival outcomes in squamous cell lung cancer [34]. In addition, a recent meta-analysis reported that SOX2 expression presents a positive prognosis in non-small cell lung cancer [35]. Previously, SOX2 overexpression was reported to be associated with favorable prognosis in squamous cell lung carcinoma, but was correlated with poor survival in adenocarcinoma [34-36]. Notably, the poor survival associated with SOX2 expression that was reported in GI tract cancer mostly pertained to adenocarcinoma [37-39]. Cervical cancer consists of squamous cell carcinoma followed by adenocarcinoma and our data comprised 80.7%
squamous cell carcinoma and 14.8% adenocarcinoma. Further research is required to clarify the prognostic significance of SOX2 in cervical cancer and variation in prognosis according to cell type.

c) Wen Li et al., Plos One (March 2015) Reported that both nuclear OCT4A and cytoplasmic OCT4B were over expressed in cervical cancer.

We have revised and added references in the discussion section as follows:

[On page 11, Discussion, Line 227-234]
In this study, OCT4 protein was observed clearly in the nucleus and partially in the cytoplasm. Similar to our findings, OCT4 has been reported in the cytoplasm as well as in the nucleus in previous studies [24, 25]. This staining pattern may arise from the presence of an OCT4 isoform. OCT4 is known to have two isoforms, OCTA and OCTB. OCT4A is observed in the nucleus and OCT4B is observed in the cytoplasm in prostate and cervical cancer [24, 26]. Because OCT4 is a transcriptional regulator, the active form of OCT4 is always located in the nucleus. For this reason, we focused our automated digital image analysis on OCT4 protein expression in the nucleus only.

2. Table 3, p-value for the association between OCT 4 and SOX 2 in cancer is not significant. Need to be justified.

We have revised and added references in the discussion section as follows:

[On page 13, Discussion, Line 270-287]
Premalignant cervical lesion demonstrated significant correlation between OCT4 and SOX2, while malignant lesion did not present an association between OCT4 and SOX2. The lack of a correlation between OCT4 and SOX2 in malignant lesions has not been explained clearly because OCT4 and SOX2 are known to work cooperatively and self-regulate themselves via the OCT4/SOX2 complex in embryonic stem cells [6, 8]. However, in the current cancer tissue samples, OCT4 and SOX2 were associated with opposite effects on survival and lose their association in cervical cancer, as well. Similar to our results, no correlation between OCT4 and SOX2 was reported in cervical cancer [23]. In addition, Li et al. also reported that OCT4 and SOX2 were not co-expressed and also showed different survival outcomes in lung cancer tissue samples [40]. Furthermore, overexpression of SOX2 inhibited the activity of OCT4 promoter in embryonal carcinoma cells [41]. OCT4 and SOX2 are known to function cooperatively through the OCT4/SOX2 complex, but OCT4, SOX2, and Nanog have been reported to form individual complexes with nucleophosmin to control stem cell fate determination [42]. In previous study, we also observed a similar phenomenon that Nanog expression in precancerous cervical tissue was correlated with Tcl1a and pAkt but this relationship lost in cancerous tissue [43].
Considering previous results and our contradictory survival data, OCT4 and SOX2 might function independently or inhibit activity during tumor progression, and eventually lose their connection in cervical cancer.

**Minor Essential Revisions:**
3. Fig 1 must be of higher objective magnification for the understanding of localization.

*We changed the Fig 1 and revised the figure legend as follows:*

![Images of OCT4 and SOX2 expression in cervical cancer tissues](image)

**Figure 1** OCT4 and SOX2 expression in formalin-fixed, paraffin-embedded cervical cancer tissues. Representative immunohistochemical image of OCT4 negative (A) and positive (B), SOX2 negative (C) and positive (D). *Insets show high magnification of areas indicated with boxes.* Scale bar: 100 μm.

**Response to Reviewer 3**

Minor Essential Revisions: There are a couple of typos such as, Background section, page 4, line 59: Correct "Radiation therapy is a widely employed" as "Radiation therapy is widely employed" Background section, page 4, line 68: Correct "are difficult eradicated, and" as "are difficult to eradicate and"

*We have carefully read and corrected the manuscript.*