Author’s response to reviews

Title: Nuclear expression of Rac1 in cervical premalignant lesions and cervical cancer cells

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Author’s response to reviews: see over
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Professor Sophie F Derchain
Journal Editorial Office
BioMed Central

Dear Professor Derchain,

I would like to thank you for your email dated 16/02/2012, concerning the manuscript: *Nuclear expression of Rac1 in cervical premalignant lesions and cervical cancer cells* (MS: 2897957275827158). In response to your kind email, we are now submitting a new version of the manuscript, in which we have improved the discussion section, according to reviewer #1 comments. Our new version of the manuscript incorporates the changes bolded in the text. Below, you will find a point-by-point response to the reviewer’s concerns.

We really appreciate the suggestions of both reviewers since they greatly improved our manuscript. We hope that you would now find our paper suitable for publication in your important Journal.

Sincerely yours,

Dr. Eduardo Castañeda Saucedo
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Point 1: Discussion as to why the Rac inhibitor does not selectively inhibit proliferation of the cervical cancer cells is warranted.

To answer to reviewer concern on the discussion about the non-selective effect of the Rac1 inhibitor on the proliferation of cervical cancer cells, we have improved the second paragraph of the discussion section; the previous paragraph was replaced by the following one:

“Rac1 has a nuclear localization signal (NLS) [24], and it has been recently shown that the importin Karyopherin alpha 2 (KPNA2) mediates Rac1 nuclear import trough the interaction with its NLS, and that KPNA2-mediated nuclear import of Rac1 requires Rac1 activation [25]. Here we show that the nuclear localization of Rac1 in C33A and SiHa cells is not affected by treatment with the Rac1 inhibitor NSC23766. These data indicate that in these cells, the presence of Rac1 in the nucleus is not dependent on its activation. Michaelson et al. (2010) showed that Rac1 translocates to the nucleus during the G2 phase of the cell cycle, and that targeting an active form of Rac1 to the nucleus promotes cell proliferation [26]. We found that chemical inhibition of Rac1 reduces the proliferation of cervical cancer cell lines C33A and SiHa, as well as that of non-tumorigenic HaCat cells. In HaCat cells, in which Rac1 is localized to the cytoplasm, chemical inhibition of Rac1 may reduce its nuclear translocation during the G2 phase of the cell cycle, resulting in a reduction in cell proliferation. Buongiorno et al. (2008) showed that an inactive form of Rac1 is present in the nucleus of colorectal cancer cells, where it associates with the transcription factor TCF-4 [27]. Interestingly, these authors demonstrated that activation of the Wnt signaling pathway induced the nuclear translocation of Tiam1, a Rac1-specific activator, in a complex with beta-catenin, and that once in the nucleus a beta-catenin/Tiam1/TCF4/Rac1 complex can be formed, resulting in the activation of Rac1 and transcriptional activation of Wnt target genes [27]. Activation of the Wnt signaling pathway plays an important role during cervical cancer progression [28, 29]; therefore nuclear Rac1 may cooperate with this pathway to stimulate proliferation of cervical cancer cells. We found that chemical inhibition Rac1 in C33A and SiHa cells, in which Rac1 localizes both to the cytoplasm and the nucleus, impairs proliferation without affecting Rac1 nuclear localization. In these cells, inactivation of the nuclear pool of Rac1 may impair the interaction of Rac1 with nuclear proteins such as TCF4 and beta-catenin, resulting in a reduction in the expression of proliferation-related genes and therefore the reduction in cell proliferation. However, Rac1 can also regulate proliferation through the activation of cytoplasmic signaling pathways such as NF-kB [30], MAPK [31], Jak/Stat [32] and Wnt [33] pathways. Therefore, it is possible that inhibition of the cytoplasmic pool of Rac1 in both cervical cancer-derived and non-tumorigenic cells may result in a reduction of cell proliferation, independently of Rac1 nuclear functions. Altogether, these data suggest that nuclear Rac1 may play an important role in regulating cell proliferation and gene expression in cervical cells, and that the presence of Rac1 in the nucleus of cervical epithelial cells from pre-malignant lesions may contribute to cancer progression.”
Point 2: Additional discussion as to potential mechanisms of Rho-GTPases in transformation would strengthen this manuscript

In order to answer to this concern, we added the following sentences at the end of the third paragraph of the discussion section:

“Experimental evidences indicate that Rho GTPases play a role in cellular transformation. It has been shown that Rac1 and its activator Tiam1 are required for Src-induced transformation [37]. Similarly, it has been demonstrated that Rac1 and Cdc42 are necessary for H-Ras-induced transformation, although overexpression of constitutively active forms of Rac1 or Cdc42 is not sufficient for cellular transformation [38]. It has also been shown that RhoA overexpression can induce pre-neoplastic transformation of primary mammary cells [39]. These data suggest that overexpression of Rho GTPases in SILs may cooperate with other signaling pathways to promote tumor progression.”