697 Bcl-2 functionally compensates for down-regulation of CHIP and protects cancer cells from cell death

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Background: Carboxy terminus of Hsp70p-Interacting Protein (CHIP) is a ubiquitin E3 ligase. CHIP adds ubiquitin to misfolded and excess proteins to induce protein degradation by proteasome pathway. Namely, CHIP controls protein quality and maintains cellular homeostasis. In our previous study, we discovered that CHIP suppresses breast cancer cell growth and metastasis. We clarified the mechanism underlying metastasis pathway. In contrast, the suppression of tumour growth by CHIP is little understood. In our experiments, we generated CHIP knocked-down MCF-7 cells by RNA interference (RNAi) (CHIP KD cells). In CHIP KD cells, we found that the levels of B-cell lymphoma 2 (Bcl-2) expression was up-regulated. Bcl-2 is known as an anti-apoptotic protein. Then we hypothesized that up-regulation of Bcl-2 promotes anchorage independent cell growth, and it may cause the enhancement of tumour growth in CHIP KD cells. Therefore, we focused on Bcl-2 and tried to elucidate the mechanism how to regulate Bcl-2 expression by CHIP.

Materials and Method:

1. Bcl-2 and CHIP were double knocked down in MCF-7 cells by RNAi. Using this cell lines, soft-agar colony formation assay was performed.
2. Bcl-2 transcriptional activity was examined in CHIP KD cells by nuclear Run-on assay.
3. CHIP expression was recovered in CHIP KD cells by adenovirus infection. Then the protein level of Bcl-2 was examined.
4. CHIP was knocked down by RNAi in MCF-7 cells. Then, the number of colonies was counted. Besides, the same examination was performed in Bcl-2 KD or over-expressed MCF-7 cells.
5. Bcl-2 expression level in MCF-7 cells was measured by flow cytometry.

Results:

1. Bcl-2 was a major factor to enhance anchorage independent cell growth by CHIP KD.
2. CHIP KD cells, Bcl-2 transcription was up-regulated.
3. Bcl-2 expression was not changed, when CHIP expression was recovered in CHIP KD cells.
4. CHIP KD induced cell death and it was suppressed by Bcl-2.
5. Bcl-2 expression level was heterogeneous in MCF-7 cells.

Conclusion: From our data, it is suggested that cellular protein homeostasis is disrupted and cell death is induced when CHIP expression is suppressed. However, portion of cancer cells that highly expresses Bcl-2 may be able to survive, because high expressing Bcl-2 functionally compensates for down-regulation of CHIP. Finally, we hypothesized that high Bcl-2 expressing cells become a majority of cellular population and they exhibit enhanced anchorage independent cell growth.

698 Nucleolar protein MYBBP1A is required for nucleolar stress-induced p53 acetylation

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The tumour suppressor protein p53 responds to a diversity of stresses and regulates many target genes whose products induce cell cycle arrest, apoptosis, senescence, and DNA repair. DNA damage is widely believed to activate p53 as a transcription factor through post-translational modifications such as phosphorylation, ubiquitination, and acetylation. Phosphorylation of p53 in response to DNA damage is mediated by various kinases. phosphorylation of Ser15 in p53 greatly increases its ability to interact with p300 and CBP. The phosphorylation-dependent interaction of histone acetyltransferases p300 with p53 drives p53 acetylation. Previous reports show that p53 acetylation is indispensable for p53 activation. Recently, a number of external and internal insults were shown to induce nucleolar stress by disrupting nucleolar structure. The impairment of nucleolar function stabilizes and activates p53. However, the mechanisms underlying this regulation are still unclear.

Previous report showed that flux of 489 endogenous nucleolar proteins and nearly 300 proteins translocate from the nucleolus to the nucleoplasm following nucleolar disruption (Andersen et al., Nature, 2005). From these 300 proteins, we excluded 200 based on their known function and selected 100 candidates for investigation. We then generated siRNAs against mRNAs for these candidate proteins and examined the effect of each siRNA on both the acetylation status and quantity of p53 proteins in cells that had been treated with TIF-IA siRNA to increase p53 acetylation.

We show that nucleolar disruption induces acetylation and accumulation of p53 without phosphorylation. Recent evidences demonstrated that p53 acetylation is an indispensable event for p53 activation. Using siRNA library, we screened nucleolar proteins involved in acetylation of p53, and identified MYBBP1A. Nucleolar disruption led to translocation of MYBBP1A from nucleolus to nucleoplasm. MYBBP1A then binds to p53 and facilitates the complex formation among p53 and p300 to induce p53 acetylation. MYBBP1A depletion significantly abrogated p53 activation and apoptosis induced by nucleolar disruption.

MYBBP1A is a signal transducer of nucleolar stress, which can directly bind to p53, bypass phosphorylation step, and induce acetylation and accumulation of p53.

699 The effects of ER alpha ligands on breast cancer metastasis

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Background: Breast cancer is typically a hormone-dependent tumour; estrogen may increase breast cancer proliferation by binding to estrogen receptor, ER alpha. ER alpha is a valuable prognostic factor for early-stage breast cancer. ER alpha-negative cancers, however, typically exhibit higher histological grades than ER alpha-positive cancers during progressive stages. In contrast, TGF-beta acts as a tumour suppressor early in tumour development, whereas it promotes invasion and metastasis at later stages. These biphasic effects of ER alpha and TGF-beta have been well known, however the molecular mechanisms remain poorly understood.

Materials and Methods:

1. MCF-7 cells were treated with ER alpha ligands, and then examined pS2 (target gene for ER alpha) or PAI1 gene (target gene for TGF-beta signaling) expression by real-time RT-PCR.
2. The effects of ER alpha ligands on ER alpha and phosphorylated Smads Protein levels were examined by Western Blotting.
3. Invasive potentials of MCF-7 cells were tested with a Matrigel-coated transwell assays in the absence or presence ER alpha ligands.

Results and Conclusion: We found that ER alpha, which is the prognostic factor of breast cancer, inhibits TGF-beta signaling. This inhibition of TGF-beta signaling is mediated by inducing the degradation of signal transducer Smad proteins in an estrogen-dependent manner. ER alpha also suppressed the breast cancer metastasis in a mouse model which evaluates cancer metastatic behavior for 6 weeks. In addition, we showed that the inhibitory effects of ER alpha expression on TGF-beta signaling were abrogated by the treatment with pure antagonist ICI182,780, which induces rapidly ER alpha protein degradation in MCF-7 cells. The ER alpha-dependent inhibition of TGF-beta signaling was not changed by the treatment with tamoxifen, which is one of the selective estrogen receptor modulators. The migratory or invasive potential of MCF-7 cells were also enhanced by ICI182,780 treatment, but not affected by tamoxifen, suggesting that the clinically treatment with ICI182,780 may induces breast cancer metastasis.

Taken together, our study indicates that breast tumour invasiveness and metastasis are inhibited by estrogen, enhanced by ICI, and not affected by tamoxifen through the regulation of TGF-beta signaling. Now we are exploring a new compound which downregulates both of tumour growth and metastasis of breast cancer.

700 BORIS and its paralogue CTCF exhibit similar biological functions

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Background: BORIS, a paralogue of the multifunctional, multivalent 11 zinc finger protein CTCF, is distinguished by its aberrant expression in both cancer cell lines and primary tumours of multiple origins. Whilst CTCF has been characterised as a putative tumour suppressor, BORIS has previously been regarded as a potential oncogene. The aim of this study was to further define the roles of CTCF and BORIS in carcinogenesis, both *in vitro* and *in vivo*.

Material and Methods: A tet-dependent multicistronic lentiviral system was constructed, allowing the regulated expression of either BORIS or CTCF in human and mouse primary cells and cancer cell lines. Assays were performed *in vitro* to assess the influence of BORIS and CTCF on cellular processes, including colony formation, proliferation, cell cycle and apoptosis. Bioluminescence imaging of an orthotopic tumour model in NOD-SCID mice was employed to determine the roles of BORIS and CTCF in tumour growth *in vivo*.

Results: Surprisingly, both BORIS and CTCF expression mediated a significant decrease in cell proliferation and clonogenic capacity, suggesting that BORIS may be acting as a tumour supressor. In asynchronous cells over-expressing CTCF, decreased cycling led to a significant increase in the percentage of cells in G0/G1. A similar trend was observed in BORIS over-expressing cells. In a UV damage induced model of apoptosis, BORIS mediated pro-apoptotic effects in primary cells, and CTCF exhibited a similar trend consistent with our previous reports. Finally, using bioluminescence imaging we demonstrate for the first time, the tumour suppressor role of CTCF in breast cancer cells *in vivo*. The growth of BORIS over-expressing tumours