TNF-alpha induced junctional modulation enhances response to radiation in Caco-2 cells

Yeonjoo Moon, Hyein Ahn, Jina Park, Byung Ryong Lee, Jaeyoun Yi* and Eunkyung Chung
*Department of Biomedical Science, Hallym University, *Lab. of modulation of radiobiological response, KIRAMS*
chungek@hallym.ac.kr

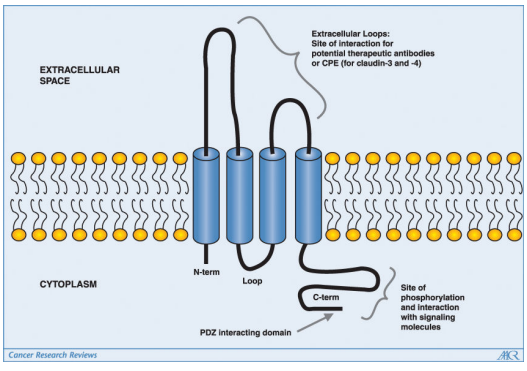
1. Introduction

The Adhesion molecules mediated cell-cell and cell-matrix interactions are essential for variety of physiological and pathological processes including maintenance of normal tissues integrity as well as tumor development and progression [1, 2].

Cell-cell interaction is initiated by interactions of tight junctional proteins with neighboring cells. Tight junctions govern the paracellular permeability of endothelial and epithelial cells. Aberrations of tight junction formation are an early and key event during vascular spread cancer and inflammation [3, 4].

TNF-alpha plays an important role in the intestinal inflammation by increase of intestinal epithelial tight junction permeability. It has been reported that TNF-alpha-modulated intestinal epithelial tight junction barrier is mediated by myosin light-chain kinase protein expression through NFk-B activation [5, 6]. However, the alterations of tight junctional proteins involved in the TNF-alpha-induced increase of intestinal TJ permeability remain unclear.

Claudin is essential to the formation and maintenance of tight junction (TJ) and has been identified 24 members so far. Claudin-1, 3, 4, 6, 10 and 16 have been shown altered in various cancers and they may have important roles in cell survival, motility, and invasion of cancer cells. However, the functions of these proteins in tumorigenesis and inflammation are still being elucidated [7, 8].



In the present study, we attempted to elucidate of TNF-alpha induced tight junctional modulations in Caco-2 cells. In addition, we tried to discriminate physiological roles of tight junctional modulations in tumor cell survival.

2. Results

2.1. TNF-alpha induces clathrin-mediated endocytosis of claudin-4 in Caco-2 cells

To elucidate molecular mechanisms underlying TNF-alpha induced inflammation in Caco-2 cells, we examined claudin family and surface-biotinylation was performed to clarify functional claudins that participate in TJ formation. We found that claudin-4 is a most abundantly expressed claudin member in Caco-2 cells and administration of TNF-alpha led surface localization of claudin-4 to the endosomal compartments in the presence or absence of cycloheximide. Moreover, the hypotonic reaction prevented TNF-alpha induced endocytosis of claudin-4, suggesting that endocytosis is clathrin-mediated.

2.2. Ionizing radiation induced increased cell death in TNF-alpha-treated Caco-2 cells

In order to determine alterations of claudin-4 influences on tumor cell survival, we performed clonongenic assay in the presence or absence of TNF-alpha. We found there was no difference in the numbers of colony. However, Caco-2 cells containing alterations of claudin-4 and increased barrier permeability by TNF-alpha treatment showed enhanced responses to radition-induced injury. The numbers of colony were significantly decreased in TNF-alpha treated Caco-2 cells in which alterations of claudin-4 were found. In conclusion, our results indicated that the modulation of claudin-4 integrity is a possible target to enhance response to radiation in human adenocarcinomas.

3. Conclusion

It has been reported that changes of adhesion molecule expression and function after exposure to ionizing radiation. Consequently, modulation of adhesion molecules by radiation may have a signaling, radioresistance, metastasis, angiogenesis, carcinogenesis, immune response, inflammation and fibrosis [9, 10].

However, our results indicated that modulation of adhesion molecules by inflammatory stimulus may have a function in controlling cellular response to radiation. Therefore, we suggest that the interactions of radiation with adhesion molecules could have a major target in developing new strategies to increase the efficacy of radiotherapy.

4. Acknowledgments

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD, Basic Research Promotion Fund) (KRF-2006-003-C00220) and Korea Science and Engineering Foundation (KOSEF-M20706000005-07B0600-00510).

REFERENCES

[1] R.G. Bauna, T.Y. Eng, C. R. Thomas, Adhesion molecules in radiotherapy, Radiat Res, Vol. 166, No. 6, pp. 819-831, 2006.
[2] M. A. Bewick, R.M. Lafrenie, Adhesion dependent signaling in the tumour microenvironment: the future of drug targeting, Curr Pharm Des, Vol. 12, No. 22, pp. 2833-2848, 2006.
[3] S. Angelow, A. S. Yu, Claudins and paracellular transport: an update, Curr Opin Nephrol Hypertens, Vol. 16, No. 5, pp. 459-464, 2007.
[4] P. B. Soeters, M. D. Luyer, J. W. Greve, W. A. Buurman, The significance of bowel permeability, Curr Opin Clin Nutr Metab Care, Vol. 10, No. 5, pp. 632-638, 2007.
[5] T. Y. Ma, M. A. Boivin, D. Ye, A. Pedram, H. M. Said, Mechanism of TNF-alpha modulation of Caco-2 intestinal epithelial tight junction barrier: role of myosin light-chain kinase protein expression, Am J Physiol Gastrointest Liver Physiol, Vol. 288, No. 3, pp. G422-430, 2005.
[6] D. Ye, I. Ma, T. Y. Ma, Molecule mechanism of TNF-alpha modulation of intestinal epithelial tight junction barrier, Am J Physiol Gastrointest Liver Physiol, Vol. 290, No. 3, pp. G496-504, 2006.
[7] P. J. Morin, Claudin proteins in human cancer: promising new targets for diagnosis and therapy, Cancer Res, Vol. 65, No. 21, pp. 9603-9606, 2005.
[8] K. J. Hewitt, R. Agarwal, JP. J. Morin, The claudin gene family: expression and neoplastic tissues, BMC Cancer, Vol. 6, No. 186, pp. 1-8, 2006.
[9] K.N. Syrigos, A. J. Karayiannakis, Adhesion molecules as targets for the treatment of neoplastic diseases, Curr Pharm Des, Vol. 12, No. 22, pp. 2849-2861, 2006.
[10] R.M. Lafrenie, C. A Buckener, M. A. Bewick, Cell adhesion and cancer: is there a potential for therapeutic intervention? , Expert Opin Ther Targets, Vol. 11, No. 6, pp. 727-731, 2007.