PO009

Validation of HER2 Expression in Cell Lines by Immunofluorescence and SISH

Jong Han Yu, Sae Byul Lee, Heeseung Park, Guiyun Sohn, Jisun Kim, Hee Jeong Kim, Beom Seok Ko, Jong Won Lee, Byung Ho Son, Sei-Hyun Ahn*

Department of Surgery, ASAN Medical Center, Korea

Background/Purpose: Circulating tumor cell has been studied for clinical meaning in many cancers including breast cancer. In breast cancer, it has also been investigated for understanding the heterogeneity of breast cancer. Particularly, discordance of human epidermal growth factor receptor (HER2) expression between primary tumor tissue and circulating tumor cell is debate for clinical significance and treatment for anti-HER2 therapy. Therefore we validated HER2 expression level by checking the immunofluroscence staining which used by detecting the HER2 -stained circulating tumor cell.

Methods: We used 6 cell-lines (MCF-7, BT20, MDA-MB 468, ZR-75-1, BT474 and SK-BR-3). All cell-lines were cultured according to the supplier's protocols (American Type Culture Collection). In order to immunofluorscense staining, all cells were attached to slide and fixed followed by staining, for which was done by using antibodies HER2, CD45 and 4',6-diamidino-2-phenylindole (DAPI). And then SMART Cytogen viewer software program ver 3.28 (CytoGen, Seoul, Korea) was used for the measurement of intensity of HER2 positive images. At the same time, all cell lines of the amplification of HER2 gene were checked.

Results: In HER2-expressed SKBR3 and BT474, HER2 average intensity by using SMART Cytogen viewer software program ver 3.28 were 241.4 and 221.28 respectively and the silver in situ hybridization (SISH) result were 12.7 and 12.15. In HER2-negative cell lines such as ZR-75-1, BT20, MCF-7, MDA-MB 468, HER2 average intensity were 139.7, 152.12, 90.2 and 36. And their SISH results were 1.56, 1.4, 1.03 and 1.13. Therefore HER2 average intensity must be over 200 if the HER2 expression is positive by immunofluorescence using SMART Cytogen viewer software program ver 3.28.

Conclusion: This study showed the feasibility of immunofluorescence staining on HER2 protein compared with SISH. Using this method, we will be able to calibrate the HER2 status of circulating tumor cell more precisely.