103P Enhancing NY-ESO-1 antigen expression in lung cancer cells through gene hypomethylation using 5-Aza-2′-deoxycytidine

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Background: NY-ESO-1 is a highly immunogenic cancer-testis antigens and it is a potential candidate for immunotherapy. Cellular expression of NY-ESO-1 gene is heterogeneous depending on the demethylation status of its promoter. Exposure to 5-aza-2'-deoxycytidine (5-aza-CdR) has been reported to enhance NY-ESO-1 protein expression. Our aim is to study the effect of 5-aza-CdR on NY-ESO-1 expression in lung cancer cell lines.

Methods: Three human lung cancer cell lines were treated with 5μM of 5-aza-CdR. NY-ESO-1 protein expression was analyzed using cellular ELISA. NCI-H1975 and NCI-H522 were tested for their dose response to increasing doses of 5-aza-CdR (2.5 to 10μM). Expression of NY-ESO-1 mRNA was assessed using qRT-PCR. Cellular ELISA, western blot (WB) and flow cytometry (FACS) techniques were used to assess NY-ESO-1 protein expression. Epigenetic modifications of CpG islands in NY-ESO-1 gene were analyzed using bisulfate sequencing. The proteomic profiling of the cells was carried out using label-free mass spectrometry analysis.

Results: NCI-H1975 and NCI-H522 cells had significantly enhanced expression (11 and 1.6 folds) of NY-ESO-1 protein after exposure to 5μM of 5-aza-CdR. Cellular ELISA showed a dose-dependent increase of NY-ESO-1 protein in both cell lines with the highest expression for NCI-H1975 (16 folds vs. control). qRT-PCR data showed a dose-dependent increase in NY-ESO-1 mRNA expression in both cells, with peak expression in NCI-H1975 cells (128 folds) at 10μM of 5-aza-CdR. FACS and WB analysis revealed increased protein expression with increasing concentration of 5-aza-CdR. Moreover, the CpG islands in the NY-ESO-1 gene were significantly hypomethylated in treated cells especially at 5μM. Proteomic profiling resulted in identification of various proteins with differential expression and functions.

Conclusions: We have shown that treatment with 5-aza-CdR enhances the expression of epigenetically silenced NY-ESO-1 antigen in lung cancer cells and this should trigger T cells immune responses against the NY-ESO-1 antigen resulting in tumor suppression. Further in vivo studies are required to apply such treatment to be as an innovative immunotherapeutic approach to treat lung cancer patients.

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