ABSTRACT

Classical Hodgkin lymphoma is a type of malignancy that originates from abnormal B lymphocytes. It is characterized by bi and multi-nucleated Hodgkin and Reed-Sternberg (HRS) cells. An important hallmark of HRS cells is overexpression of CD30 and PD-L1 (Programmed cell death protein 1 ligand) on the cell surface. Overexpression of PD-L1 plays an important role in proliferation and metabolism of tumor cells. The modern immunotherapy using anti-PD-1/PDL1 antibodies enhances the response of T cell to cancer cells and ultimately blocks the proliferation of HRS cells. For our study we used the three certified HRS cell lines: KM-H2, L-1236, and L428. After 3-5 days, cells from all harvested lines subjected for Western blot analysis with antibodies: anti PD-L1, EZH2 and SWI/SNF subunits to verify in which line the PD-L1 was overexpressed. The immunoprecipitation was performed on L-1236 HRS cells followed by mass spectrometry analysis. The data obtained from this analysis were further confirmed by other methods like Yeast two hybrid system. Furthermore, we performed chromatin immunoprecipitation (ChIP) on L-1236 cHL line using antibodies: anti SWI/SNF (BAF 155), PRC2 complex (EZH2), PD-L1 as well as methylated and acetylated Histone H3 on CD274 promoter region. Furthermore, the MTT assay was performed after the treatment of cells from L1236 cell line with EZH2 inhibitor (EPZ-6438).

We investigated the PD-L1 translocation into cell nuclei and identified its potential nuclear partners in L-1236 cell line. After the mass spectrometry analysis, we found that, PD-L1 may interact in the nucleus with the splicing machinery and thus regulate the RNA posttranscriptional alternative processing. Noteworthy, we subsequently found that the subunits of SWI/SNF (BAF 155), PRC2 complex (EZH2), PD-L1 and methylated and acetylated H3 are located at the same position i.e. (-606) on the promoter region of *CD274* gene that encodes PD-L1 protein. We also analyzed and confirmed the PD-L1 interaction with BAFF155, FUS, SNAIL, and SLUG after Yeast two hybrid assay, all these findings together provide a clue about their involvement in regulating PD-L1 expression and PD-L1 itself in a positive feedback loop manner as well as PDL1 function in the nucleus. The observations after EZH2 inhibitor in HRS cells revealed that EZH2 is not an essential component to be targeted for therapeutic purposes although they are present at PD-L1 loci but there are some additional mechanisms and factors involved in regulation of PD-L1 overexpression that needs further exploration.