Poster #: 1215

XXI IWCLL International Workshop on CLL

Expression and signalling patterns of CD180/MD1 and CD150 in Chronic Lymphocytic Leukaemia (CLL) cells and MEC 1 cell line

Tamar Tsertsvadze [1,3], Kurtis Edwards [2], Sarah John-Olabode[2], Irina Datikashvili-David [4], Ani Bilanishvili [1], Peter M Lydyard [2,3], Nina Porakishvili [2]

1 Javakhishvili Tbilisi State University, Georgia;
2 University of Westminster, London, UK;
3 University of Georgia, Tbilisi, Georgia;
4 Aversi Clinic, Tbilisi, Georgia;

OBJECTIVES

This study aimed to investigate the surface and intracellular expression patterns of CD180, MD-1, and CD150 in primary chronic lymphocytic leukemia (CLL) cells and the MEC-1 cell line. We further assessed their association with AKT and p38MAPK signaling responses and evaluated their potential prognostic relevance in the context of CLL.

CONCLUSIONS

Downregulation of surface and cytoplasmic expression of MD1 is associated with a reduction in CD180 surface expression in CLL cells, which could impair CD180-mediated signaling and thus contribute to disease progression. This finding supports the hypothesis that MD1 plays a pivotal role in regulating CD180 availability and signaling capacity, particularly in the context of CLL.

The inverse relationship between CD150 expression and CD180-mediated AKT signaling in CLL highlights the complex regulatory mechanisms involved in CLL cell survival and proliferation. Our results suggest that CD150 expression may modulate the signaling pathways activated by CD180, influencing the heterogeneity of CLL cell responses.

Importantly, CD150 stimulation alone was also capable of inducing distinct signaling profiles. This suggests that CD150 functions as a co-receptor and an independent modulator of intracellular signaling. CD150-mediated signaling may depend on the expression levels of CD180, further highlighting the dynamic interplay between these receptors.

These findings are consistent with previous research on the CD180/CD150/MD1 axis and offer further validation of the importance of these molecules in CLL pathophysiology.

INTRODUCTION

We have previously shown that, differently from normal peripheral blood (PB) and tonsillar B cells, expression of the CD180 toll-like receptor is highly heterogeneous on PB and lymph node chronic lymphocytic leukaemia (CLL) cells [1-3]. The MD-1 satellite molecule, essential for CD180 expression on the cell surface, could play a role in this heterogeneity [3]. Importantly, we have shown that CD180 could hold prognostic value, since its high expression is associated with early-stage disease, M-CLL and superior overall survival [1,3]. The ability of CD180 to signal via pro-apoptotic p38MAPK pathway and re-wire IgM-mediated AKT survival signalling to a p38MAPK pathway [4], explains the positive association between CD180 expression and favourable prognosis. Similarly, higher expression of CD150, a member of the signalling lymphocyte activation molecule family (SLAMF), that is co-expressed with CD180 on CLL cells, has been associated with enhanced survival [5]. We therefore aimed to characterise the cell surface (CS) and intracellular (IC) expression of CD180 and MD-1, as well as the surface expression of CD150, in primary CLL cells and the CLL-derived MEC-1 cell line. Additionally, we assessed the correlation between these expression patterns with intracellular signalling.

METHODS

Peripheral blood mononuclear cells were isolated from 26 untreated CLL patients (clinic “Aversi”, care of Dr Datikashvili-David, upon informed consent) using density gradient and immunophenotyped with APC-Cy7-conjugated anti-CD180 (clone G28-8, BD), PE-conjugated polyclonal anti-MD-1, and APC-conjugated anti-CD150 (clone SLAM.4, Abcam). $CD19^+$ cells were identified using anti-CD19 FITC (BD). For intracellular expression, cells were stained with anti-CD19 FITC and anti-CD150 APC, fixed and permeabilized, and stained for anti-CD180 or anti-MD-1.

Eleven $CD180^+MD1^+$ CLL samples were stimulated with anti-CD180 and anti-CD150 for 30 minutes at 37°C, followed by staining with PE-conjugated anti-pAKT or APC-conjugated anti-p-p38MAPK (BD). Flow cytometry analysis was conducted on a NovoCyte 2060 (ACEA Biosciences). The same procedures were performed on the MEC1 cell line. MEC1 cells were synchronised by serum starvation and analysed at 0, 24, 48, and 72 hours.

RESULTS

CD180 and MD-1 expression in CLL samples was assessed as cell surface (CS) or intracellular IC+CS. As expected, permeabilization significantly increased the percentages of CD180-positive IC+CS across all samples (p=0.01, Figure1a), even in those with low CS levels, thus indicating a substantial intracellular CD180, regardless of surface expression. A similar trend was observed for MD-1 (p=0.06, Figure 1a). CD150 surface expression was heterogeneous and broadly paralleled CD180 and MD-1 patterns (Figure 1b).

Due to the heterogeneity in the CS expression of CD180 and MD-1, and to explore potential correlations, we stratified CLL samples based on surface expression of both CD180 and CD150, using a 60% positivity threshold. We found that CS and IC+CS levels of MD-1 were significantly higher in $CD180^{high}$ compared to $CD180^{low}$cases (Figure 2). Interestingly, CS MD1 and IC+CS MD1 expression was significantly elevated in $CD150^{high}$ compared to $CD150^{low}$ subset (Figure 3), suggesting a direct correlation between the expression of MD1 with both CD180 and CD150.

In MEC-1 cells, CS CD180 increased at 24h (p = 0.009) and 48h (p = 0.006), returning to baseline at 72h (p = 0.15). IC+CS CD180 peaked at 48h (p = 0.008), then declined (p = 0.003), yet remained higher than CS at 72h (p = 0.03) (Figure 4a). MD-1 peaked earlier at 24h (CS p = 0.001; IC+CS p = 0.003), then declined at 48h and 72h. IC+CS MD-1 remained higher than CS at both 24h (p = 0.007) and 72h (p = 0.003), suggesting MD-1 upregulation precedes CD180. CD150 expression remained high with slight decreases at 48h (p = 0.05) and 72h (p = 0.03) (Figure 4b).

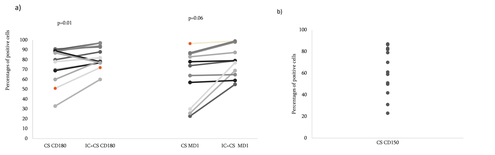
Stimulation of $CD180^+MD1^+$ CLL samples with anti-CD180 revealed distinct signaling subsets, including AKT-signalers (AKT-S), p38MAPK-signalers (p38MAPK-S), non-signalers (NS), and dual AKT/p38MAPK signalers (DS) as reported by us before (Figure 5a and b).

Notably, all cases identified as AKT-S were $CD180^{high}/CD150^{low}$, underscoring a potential link between low CD150 expression and preferred AKT signaling (Figure 6). Similar to CD180 stimulation, AKT-S, p38MAPK-S, NS, and DS subsets were observed following anti-CD150 stimulation (Figure 7a and b).

Interestingly, combined stimulation of $CD180^{high}MD\text{-}1^{high}$ $CD150^{high}$ CLL cells with anti-CD180 and anti-CD150 mAbs did not lead to any gain of function expressed by the enhanced phosphorylation of AKT or p38MAPK compared to the stimulation with sole antibodies (Figure 8).

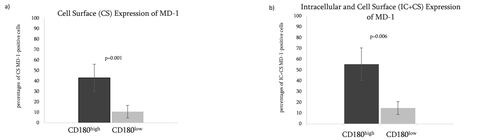
Despite high CS CD180/MD1 (Figure 4a) and CS CD150 (Figure 4b) expression at 24 hours, stimulation with anti-CD180, anti-CD150, or both did not induce AKT or p38MAPK phosphorylation (Figure 9a). At 48 hours, anti-CD150 (p=0.04) and combined anti-CD180/CD150 (p=0.017) reduced p-p38MAPK levels, while both treatments significantly increased $pAKT^+$ cells (p=0.005 and p=0.002, respectively) compared to controls (Figure 9b).

Figure 1. Cell surface and intracellular expression of CD180, MD1, and CD150 in CLL cells.



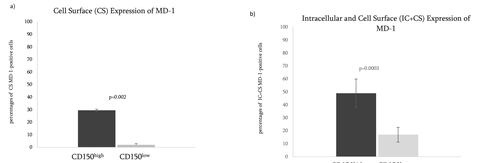
a) Cell surface (CS) and intracellular (IC + CS) expression of CD180 and MD1 in CLL cells. (CS CD180: 73.6 ± 9.5%, n = 13; IC+CS CD180: 82.0 ± 5.0%, n = 13, p = 0.01; CS MD1: 67.4 ± 12.0%, n = 10; IC+CS MD1: 78.9 ± 8.0%, n =10, p = 0.06). b) Cell surface (CS) expression of CD150 in CLL cells (CS CD150: 62.5 ± 12.4%, n = 13).

Figure 2. Cell Surface and Intracellular Expression of MD1 in $CD180^{high}$ and $CD180^{low}$ CLL subsets.



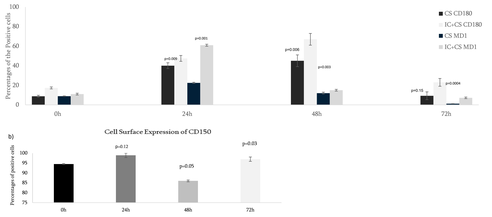
a) Cell surface expression of MD1 in $CD180^{high}$ (43.3 ± 11.0%, n = 16) and $CD180^{low}$ (10.7 ± 6.3%, n = 16) CLL subsets (p = 0.001). b) Intracellular and cell surface expression of MD1 in $CD180^{high}$ (55.5 ± 16.0%, n = 14) and $CD180^{low}$ (14.8 ± 6.0%, n = 12) CLL cells (p = 0.006).

Figure 3. Cell Surface and Intracellular Expression of MD1 in $CD150^{high}$ and $CD150^{low}$ CLL subsets.



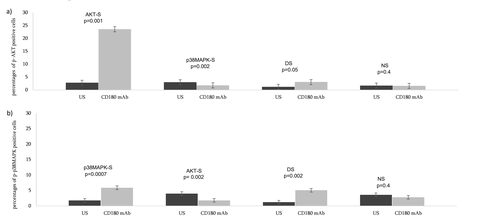
a) Cell surface expression of MD1 in $CD150^{high}$ (29.2±1.0%, n=15) and $CD150^{low}$ (2.1±0.9%, n=15) CLL cells (p=0.002). b) Intracellular and cell surface expression of MD1 in $CD150^{high}$ (49.3±11.0%, n=16) vs $CD150^{low}$ CLL cells (17.6± 5.7%, n = 16, p = 0.0003).

Figure 4. Time-dependent expression of CD180, MD1, and CD150 in MEC-1 cells.



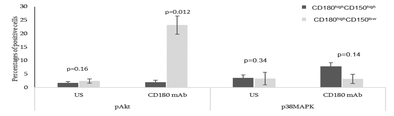
a) Surface (CS) and intracellular and surface (IC+CS) expression of CD180 and MD1 at 0h, 24h, 48h, and 72h of actively cycling MEC-1 cell culture (n = 8).. b) Cell surface expression of CD150 at 0h, 24h, 48h, and 72h of actively cycling MEC-1 cell culture (n = 8).

Figure 5. Phosphorylation of AKT and p38MAPK in CLL cells following anti-CD180 stimulation.



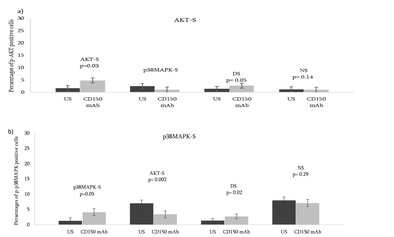
(a) AKT phosphorylation in CLL cells after anti-CD180 stimulation, shown for AKT signalers (AKT-S, n=7), p38MAPK signalers (p38MAPK-S, n=8), non-signalers (NS, n=9), and dual AKT/p38MAPK signalers (DS, n=8). (b) p38MAPK phosphorylation in CLL cells after anti-CD180 stimulation, shown for p38MAPK signalers (p38MAPK-S, n=8), AKT signalers (AKT-S, n=7), dual AKT/p38MAPK signalers (DS, n=8), and non-signalers (NS, n=7).

Figure 6. Anti-CD180 induced phosphorylation of AKT and p38MAPK in CD180highCD150high and CD180highCD150low CLL subsets.



$CD180^{high}CD150^{high}$ subset: AKT (US 2.6 ± 0.6%, CD180 mAb 1.9 ± 0.7%, n=16); p38MAPK (US 2.6 ± 1.4%, CD180 mAb 7.8 ± 0.9%, n=16). $CD180^{high}CD150^{low}$ subset: AKT (US 2.4 ± 1.6%, CD180 mAb 23.2 ± 6.7%, n=11); p38MAPK (US 4.3 ± 1.4%, CD180 mAb 2.1 ± 1.8%, n=11).

Figure 7. Phosphorylation responses of AKT and p38MAPK in CLL cells following anti-CD150 stimulation.



a) AKT phosphorylation in CLL cells after anti-CD150 stimulation, shown for AKT signalers (AKT-S, n=6), p38MAPK signalers (p38MAPK-S, n=7), non-signalers (NS, n=8) and double AKT/p38MAPK signalers (DS, n=7). b) p38MAPK phosphorylation in CLL cells after anti-CD150 stimulation, shown for p38MAPK signalers (p38MAPK-S, n=7), AKT signalers (AKT-S, n=6), dual AKT/p38MAPK signalers (DS, n=7), and non-signalers (NS, n=8).

Figure 8. Phosphorylation of AKT and p38MAPK in $CD180^{high}MD1^{high}CD150^{high}$CLL cells following single and combined stimulation (n=7).

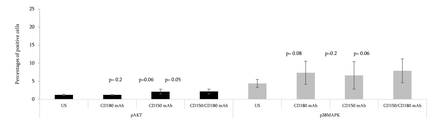
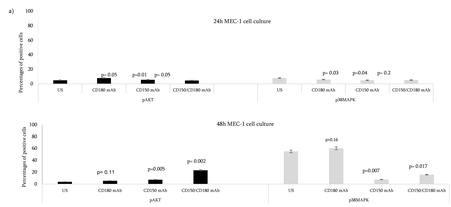


Figure 9. Phosphorylation of AKT and p38MAPK in MEC-1 cells in unstimulated cells (US), following stimulation with anti-CD180, anti-CD150 or both antibodies together.



a) 24h MEC-1 cell culture b) 48h MEC-1cell culture.

REFERENCES

1. Porakishvili *et al., Br J Haematol*,2005,131:313-319.
2. Porakishvili *et al., Br J Haematol*,2011,153:486–498.
3. Porakishvili *et al., Mol Med*,2015,21:46-57.
4. Edwards *et al., Leukaemia Research*,143, 2024.
5. Gordienko *et al., PLoS One*,2017,12(10).

ACKNOWLEDGMENTS

This work was supported by **Shota Rustaveli National Science Foundation of Georgia (SRNSFG) [Grant number FR-22-9688].**

DISCLOSURES

The authors have no relevant financial or non-financial interests to disclose

Corresponding author: Tamar Tsertsvadze
tamuna.tsertsvadze@gmail.com
LinkedIn: Tamar Tsertsvadze
WhatsApp: +995 599 18 10 23