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# TGF-β SIGNALING-MEDIATED UPREGULATION OF PODOPLANIN: A PROMISING BIOMARKER FOR DIAGNOSIS AND PROGNOSIS IN ACUTE PROMYELOCYTIC LEUKEMIA


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## INTRODUCTION

- Acute promyelocytic leukaemia (APL), distinguished by the presence of PML-RARA fusion oncoprotein, is highly curable if diagnosed early, making it a medical emergency (Grimwade et al., 2010; Ablain et al., 2013; Iland, 2019).
- Diagnosis relies on subjective bone-marrow morphology, requiring extensive experience and still could be misdiagnose as AML-M5 or AML-M2, leading to delayed treatment and fatal outcomes (Bain and Bene, 2019; Gujral et al., 2009).
- A recent study found that Podoplanin (PDPN) overexpression is uniquely associated with APL blasts and contribute to pathophysiology (Lavallee et al., 2018), however the molecular regulation of PDPN is unexplored.
- PDPN also interact with CLEC2 to activate the platelets that leads to coagulopathy and contribute to pathophysiology of the disease.
- We evaluated the expression pattern and molecular regulation of PDPN in APL and AML patients.

## AIM

The current study aim to validate whether PDPN expression through flow cytometry and real-time quantitative PCR (RQ-PCR) at diagnosis can accurately distinguish APL from AML. We also explored the putative molecular regulation of PDPN in APL.

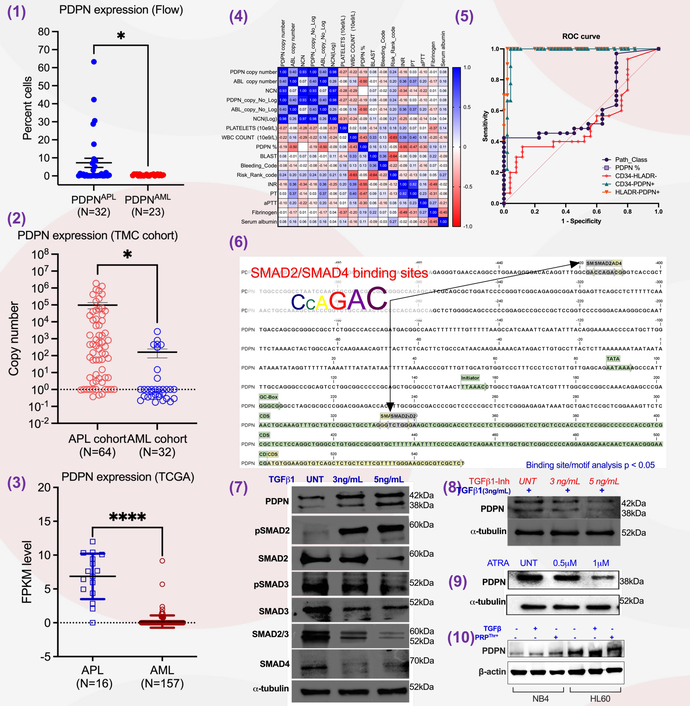
## METHOD

- PDPN, CD34, HLA/DR, etc. expression levels was measured in APL and AML patients using flow cytometry.
- For copy number analysis PDPN was cloned from HL60 cell line and TaqMan assay was used for quantification.
- Western blot analysis was performed using standard procedure.
- We used synthetic TGFβ for induction while Luspatercept for inhibition of TGFβ signaling that contribute significantly in PDPN expression.
- Statistical analysis was performed using paired t-test and regression analysis. ROC analysis was performed to evaluate the sensitivity and specificity of PDPN in APL diagnosis.

## RESULTS

- The median surface expression of PDPN in APL was 7.24% vs. 0.51% in AML **(Figure 1)**.
- RQ-PCR showed **significantly higher mean normalized copy number of PDPN in APL** (0.141) cases than in AML (0.0058) **(Figure 2)**.
- These results were consistent with the TCGA-dataset, which showed higher PDPN expression in APL patients (p=0.0124) **(Figure 3)**.
- APL patients showed significant correlations of PDPN expression with classical risk markers for APL *i.e.* high WBC count (p<0.0001)] and low platelet count (p=0.0415) as well as CD34- and HLA/DR- phenotype (p=0.038) **(Figure 4; Table I)**.
- Analysis of sensitivity and specificity revealed that PDPN alone (60.0%, 63.6%) and in combination with either CD34- (60.0%, 83.6%) or HLA/DR- (60.0%, 81.8%) had superior predictive performance for distinguishing APL from AML compared to the dual marker combination of CD34- and HLA/DR- (60.0%, 45.5%) **(Figure 5)**.
- We also investigated the molecular mechanism behind PDPN overexpression in APL cells.
- *In-silico* analysis showed SMAD2/ SMAD4 bindings on PDPN promoter **(Figure 6)**.
- APL cells treated with recombinant TGF-β (3ng/mL and 5ng/mL) increased PDPN expression (p<0.05) along with upregulation of TGFβ signalling evident by increase in pSMAD2 and pSMAD3 levels **(Figure 7)**.
- A rescue experiment with the TGF-β inhibitor, Luspatercept reduced PDPN expression, highlighting the role of TGF-β signalling **(Figure 8)**.
- ATRA treatment also results in decreased expression of PDPN (Figure 8) validating the earlier findings that PDPN expression as well as platelet aggregation decreases after ATRA treatment **(Figure 9)**.
- Platelet rich plasma activated with thrombin also upregulated PDPN similar to TGF-β mediated upregulation in APL *in-vitro* model indicating a feedback loop **(Figure 10)**.

| Parameter | AML (n=23) | Range (min, Max) | APL (n=32) | Range (min, Max) | p value (t-test) |
|---|---|---|---|---|---|
| Age mean (median) | 31 (31) | 1-56 years | 33 (39) | 8-64 years | 0.5607 |
| average Hb (g/dL) | 8.020 | 4.2-11.7 g/dL | 8.960 | 4.8-12.6 g/dL | 0.0868 |
| mean aPTT | 5.225 | 0-28.1 | 27.253 | 17.6-41.7 | 0.0000 |
| Mean PDPN | 0.509 | 0-1 | 7.239 | 0.018-63.28 | 0.0133 |
| Mean blast % | 63.381 | 22-92 | 65.813 | 0-96 | 0.7383 |
| Mean WBC count | 5.99*10^4 | 1.81*10^3- 2.3310^5 | 20.42*10^9 | 0.6-106.0*10^9 | 0.0001 |
| Platelets 10e9/L | 118.8 | 15-1045 | 36.19 | 8-121 | 0.1223 |




**PDPN expression in APL is associated with platelet activation and mediated by TGFβ signaling pathway. PDPN can potentially serve as diagnostic as well as prognostic biomarker.**

## CONCLUSIONS

- PDPN expression is more specific to APL compared to AML patients.
- PDPN in combination with CD34- and HLA/DR- have superior diagnostic accuracy.
- PDPN expression is regulated by TGF-β signalling pathway.
- Inhibition of TGF-β signalling results in downregulation of PDPN in APL.
- ATRA also results in downregulation of PDPN in APL cell line.
- Our findings suggest PDPN as a potential diagnostic biomarker. PDPN can also serve as a potential therapeutic target and putative prognostic marker in APL patients.

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