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Deregulation of protein phosphatase expression in acute myeloid leukemia

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Keywords: Acute myeloid leukemia; AML; Protein phosphatase; Protein tyrosine phosphatase; Protein Serine/threonine phosphatase; Dual-specificity protein phosphatase.

Abstract

Acute myeloid leukemia (AML) is a highly malignant disease of myeloid cell line. AML is the most frequent adult leukemia with inadequate treatment possibility. The protein phosphatases are critical regulators of cell signaling, and deregulation of protein phosphatases always contribute to cell transformation. Although many studies established a relationship between protein phosphatases and leukemia, little is known about the role of this group of proteins in AML. To address this issue, we initially identified the complete catalog of human protein phosphatase genes and used this catalog to study deregulation of protein phosphatases in AML. Using mRNA expression data of AML patients, we show that 11 protein phosphatases are deregulated in AML within 174 protein phosphatases. The GO enrichment study suggests that these genes are involved in multiple biological processes other than protein de-phosphorylation. Expression of DUSP10, PTPRC and PTPRE was significantly higher than average expression in AML, and a linear combination of DUSP10, MTMR11, PTPN4, and PTPRE expressions provides important information about disease subtypes. Our results provide an overview of protein phosphatase deregulation in AML.

Introduction

Acute myeloid leukemia (AML) is the most frequent adult leukemia that generally leads to poor outcome. In past years, a considerable progression has been made in our understanding of the molecular genetic and epigenetic basis of AML and in defining new diagnostic and prognostic markers [1]. Development of novel therapies targeting specific genetic alteration displayed promising improvement of treatment quality. However, these approaches always suffer from primary or secondary accumulation of resistance to the drug. Thus, new therapeutic targets are needed.

The reversible protein phosphorylation is the fundamental mechanism by which majority biological functions are regulated. These processes are controlled by two groups of proteins, protein kinases, and protein phosphatases. Several studies suggest the presence of more than 500 protein kinases in the mammalian genome [2-5]. However, to counter these protein kinases, much fewer protein phosphatases are existed. Due to the wide range of substrate specificity, one protein phosphatase can reverse the action of multiple kinases. The kinase-mediated substrate phosphorylation results in the activation of signaling pathways which can be turned off by phosphatase. Thus, loss of phosphatase function can contribute in uncontrolled cellular processes. Phosphatases can also activate several signaling cascades. For instance, de-phosphorylation of regulatory tyrosine residue of Src family kinases activates this family of proteins. The SH2 domain containing phosphatase, SHP2 acts as adaptor protein, thus linking components in signal transduction pathways. So overexpression of certain protein phosphatases might also have adverse cellular affects.

Recent studies describe the role of protein phosphatases in different acute leukemia subtypes. The receptor tyrosine phosphatase PTPRC displays a tumor suppressor role in T-cell acute lymphoblastic leukemia [6]. Inactivation of another receptor tyrosine phosphatase PTPRJ contributed to the FLT3-ITD-driven transformation in myeloproliferative disease [7].

Another recent study with protein tyrosine phosphatases (PTPs) suggests that a number of PTPs are abundantly expressed in AML [8]. In this study, using the complete set of protein phosphatases, we show that eleven phosphatase genes are deregulated in AML and four phosphatase genes expression signature can be used as subtype-specific marker.

Materials and methods

Searching for protein phosphatases

Publicly accessible genetic sequence databases were used to search for protein phosphatases in human genome. We first retrieved cDNA sequence of all known protein phosphatases available in UniProt database from different species. We used them as query to search for novel protein phosphatases by local BLAST. We then used phosphatase domains available in SMART and Pfam databases to build Hidden Markov Models and used them to search for additional protein phosphatases. Searches were refined using method described elsewhere [3]. These searches resulted in a complete set of non-redundant protein phosphatases.

Microarray data analysis

The microarray dataset for human AML patient samples (GSE9476, GSE5550, GSE2191, GSE12417, GSE425, and GSE1729) were retrieved from NCBI GEO site. Methods used to retrieve, scale, and analyze data were described previously [9-12]. In brief, data retrieved from NCBI GEO site were scaled using median scale normalization method. Normalized data were processed for statistical analysis.

Statistical analysis

Statistical analysis was done using GraphPad Prism 5. One way ANOVA with Bonferroni's Multiple Comparison Post Test was used to determine significance.

Results

The catalog of human protein phosphatases

To identify the role of protein phosphatases, we first set out to determine the complete set of protein phosphatases in human genome. Using methods described above, we cataloged 174 protein phosphatases. This catalog includes 65 dual specificity protein phosphatase (DSPP) genes, 12 atypical protein tyrosine phosphatase (PTP) genes, 38 classical PTP genes, 33 protein serine/threonine phosphatase (PSTP) genes, and 26 protein serine/threonine phosphatase-like (PSTPL) genes (Fig. 1A). This catalog strongly overlaps, but does differ from, the previously published set of phosphatases in human or other genomes [13, 14]. Further classification identified 21 families in 5 groups (Fig. 1B-F).

Deregulated protein phosphatase in AML

We then used our catalog to analyze the role of this class of proteins in acute myeloid leukemia (AML). Initially, a microarray dataset of AML patients and the corresponding healthy donors was analyzed for mRNA expression [15]. Iterative one-way ANOVA with Bonferroni's post-test analysis identified 11 deregulated phosphatases in AML patients (Fig. 2A). Four DSPPs including DUSP3, DUSP10, MTMR3, and MTMR11 were deregulated. While expression of DUSP3 and DUSP10 was up-regulated, expression of two myotubularin was down-regulated. Among the two deregulated atypical PTPs, PTPLA expresses a catalytically inactive phosphatase-like domain. According to OMIM, this gene is involved in Alzheimer disease and Arrhythmogenic right ventricular dysplasia. Three classical PTPs are deregulated of which PTPRC and PTPRE displayed the highest expression within the whole group. We also observed that two serine/threonine-like protein phosphatases which were further classified as metallophos were down-regulated in AML patients.

Deregulated protein phosphatases are involved in diverse biological processes

Since we observed that 11 protein phosphatases are deregulated in AML, we analyzed their biological roles using GO enrichment. We searched GO enrichment information using GOEAST. We applied Hypergeometric analysis for biological process search with the p value cutoff 0.001 for false discovery rate (FDR). Although, most of genes are predominantly involved in protein de-phosphorylation, these genes also regulate several cellular processes (Fig. 3A).

Four phosphatase gene signature in AML

To explore the relative expression of these genes in AML, we analyzed large-scale expression data. We retrieved mRNA expression data for 456 AML patients [15-19]. Analysis with 11 genes displayed differential expressions within those selected genes (Fig. 4A). Expression of DUSP10, PTPRC, and PTPRE was significantly increased compared to average expression, and MTMR11, PTPN4, CPPED1 and SMPDL3A expression were significantly decreased. Thus, we suggest that these 7 genes might play a role in AML progression. We then tested whether these genes have prognostic values. We included 5 genes to test prognostic significance, while CCPED1 and SMPDL3A genes were excluded due to the lack of sufficient expression data in different disease subtypes. We observed that the expression of individual genes did not exhibit any correlation with disease subtypes (Fig. 4B-F). However, a linear combination of DUSP10, MTMR11, PTPN4, and PTPRE provides important information about disease subtypes. Thus we calculated the relative expression (y) using the equation $y=\sqrt{(((a+b)/2-(c+d)/2)^2)}$, where a=DUSP10 expression, b=PTPRE expression, c=MTMR11 expression and d=PTPN4 expression. Result suggests that higher y value is related to M0-M4 subtypes while lower y value is related to M5 and M7 subtypes (Fig. 5A). Neither the expression of individual genes nor relative expression (y) displayed a correlation with overall survival (data not shown and Fig. 5B) suggesting that deregulated phosphatase expression might not play a role on AML patients' survival. Furthermore, no

significant difference was observed in phosphatase expression in different sexes of patients (Fig. 5C). However, we observed that the relative expression (y) displays a weak correlation with patient age (r^2<0.25) (Fig. 5D).

Discussion

Uncontrolled phosphorylation of signaling proteins contributes to malignancy. Protein phosphatases balance cellular phosphorylation levels by encountering kinases. Recent studies suggest that protein phosphatases play important roles in leukemia [20]. We analyzed expression of protein phosphatases in AML and compared with healthy donors. We observed that expression of 11 protein phosphatases is deregulated in AML.

Abundant expression of protein tyrosine phosphatases was reported in AML that further elevated in cells expressing an oncogenic FLT3 mutant FLT3-ITD [8]. Expression of DUSP6 was highly expressed in AML cells having FLT3-ITD mutation and DUSP6 depletion contributed in increased Erk activation, suggesting that DUSP6 controls FLT3-ITD downstream signaling [8]. We observed that expression of DUSP3 and DUSP10 was up-regulated in AML. DUSP10 was reported to be up-regulated in colorectal cancer and kept Erk in cytosol by direct interaction, suggesting that DUSP10 also act as adaptor protein [21]. DUSP3 controls Cyclin D1 expression in breast cancer [22]. PTPRC is the most studied phosphatase in leukemia. Increased PTPRC expression correlated with poor prognosis in AML and acute lymphoblastic leukemia (ALL) [20].

Although protein phosphatases mainly de-phosphorylate substrate proteins, this group of proteins also contributes to several biological processes. We observed that de-regulated protein phosphatases take part in T-cell receptor signaling pathways as well as regulation of immune response suggesting complexity of phosphatase-mediated signaling. Furthermore deregulated protein phosphatases displayed a correlation with AML subtype, suggesting that

phosphatase expression signature can be used in AML patient classification. This correlation was independent within patient sexes, ages, and prognosis further provides evidence that this gene signature can be used for wide range of AML patients.

Thus, we conclude that protein phosphatase expression is deregulated in AML, and relative expression (y) of 4 deregulated phosphatase genes (DUSP10, PTPRE, MTMR11, and PTPN4) will provide valuable information which can be used for patient classification.

Potential conflict of interest: The authors declared no conflict of interest.

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Figure Legends:

Fig. 1. Catalog of protein phosphatases in human genome:

The complete set of protein phosphatase was classified into five groups and twenty-one families according to phosphatase domains.

Fig. 2. Deregulated protein phosphatases in AML patients:

Deregulated phosphatase genes were identified using iterative one-way ANOVA. *, $p<0.05$; **, $p<0.01$ and ***, $p<0.001$.

Fig. 3. GO enrichment analysis of deregulated protein phosphatases in AML patients:

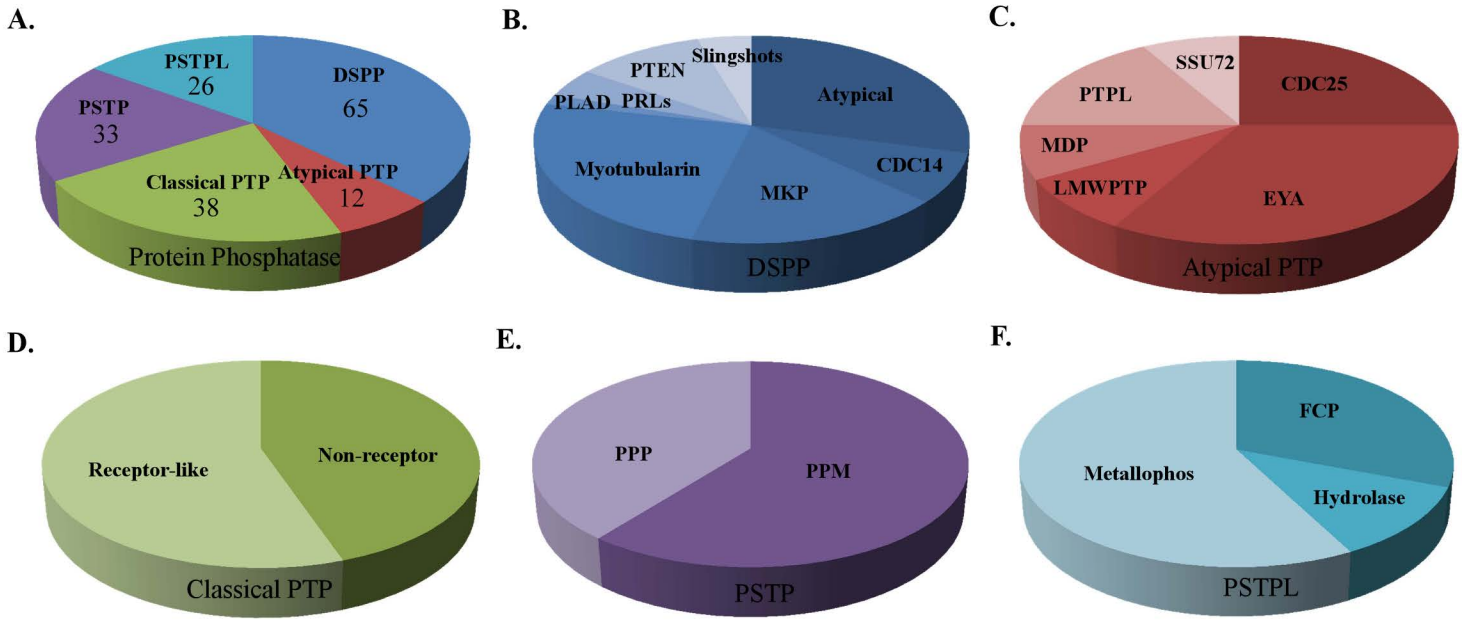
GO enrichment of phosphatase genes using GO term biological processes. Hypergeometric analysis for biological process search with the p value cutoff 0.001 for false discovery rate (FDR) was applied for GO enrichment in GOEAST.

Fig. 4. Relative expression of deregulated protein phosphatases in AML patients:

(A) Up-regulated and down-regulated phosphatase genes in 456 AML patients. (B) Relative expression of PTPRE gene in different disease subtypes. (C) Relative expression of PTPN4 gene in different disease subtypes. (D) Relative expression of PTPRC gene in different disease subtypes. (E) Relative expression of MTMR11 gene in different disease subtypes. (F) Relative expression of DUSP10 gene in different disease subtypes.

Fig. 5. Relative expression of deregulated protein phosphatases correlated with AML subtypes:

(A) Calculated relative expression (y) of phosphatase genes in different disease subtypes. (B) Correlation between calculated relative expression (y) of phosphatase genes and overall survival. (C) Individual and calculated relative expression (y) of phosphatase genes in different sexes. (D) Correlation between calculated relative expression (y) of phosphatase genes and patient age.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PSTPL
26
DSPP
65
PSTP
33
Classical PTP
38
Atypical PTP
12
Protein Phosphatase
B.
Slingshots
PTEN
PLAD
PRLs
Atypical
Myotubularin
CDC14
MKP
DSPP
C.
SSU72
CDC25
PTPL
MDP
LMWPTP
EYA
Atypical PTP
D.
Receptor-like
Non-receptor
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PPM
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FCP
Metallophos
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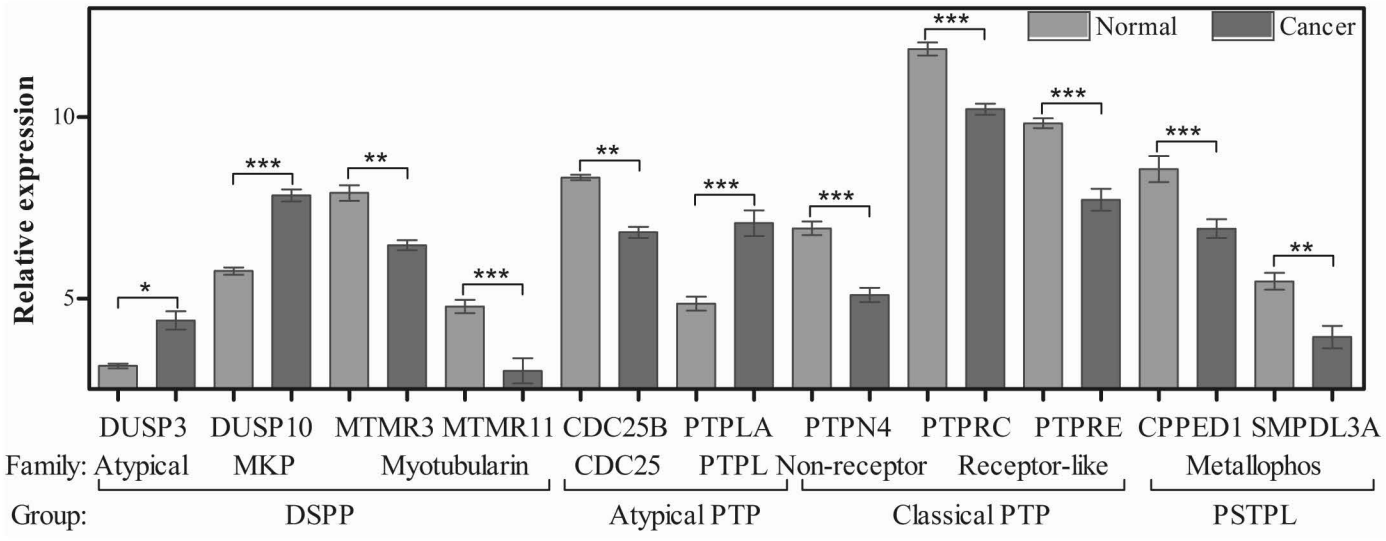
PSTPL

Figure 1

A.


Normal
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MTMR11
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PTPN4
PTPRC
PTPRE
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SMPDL3A
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Myotubularin
CDC25
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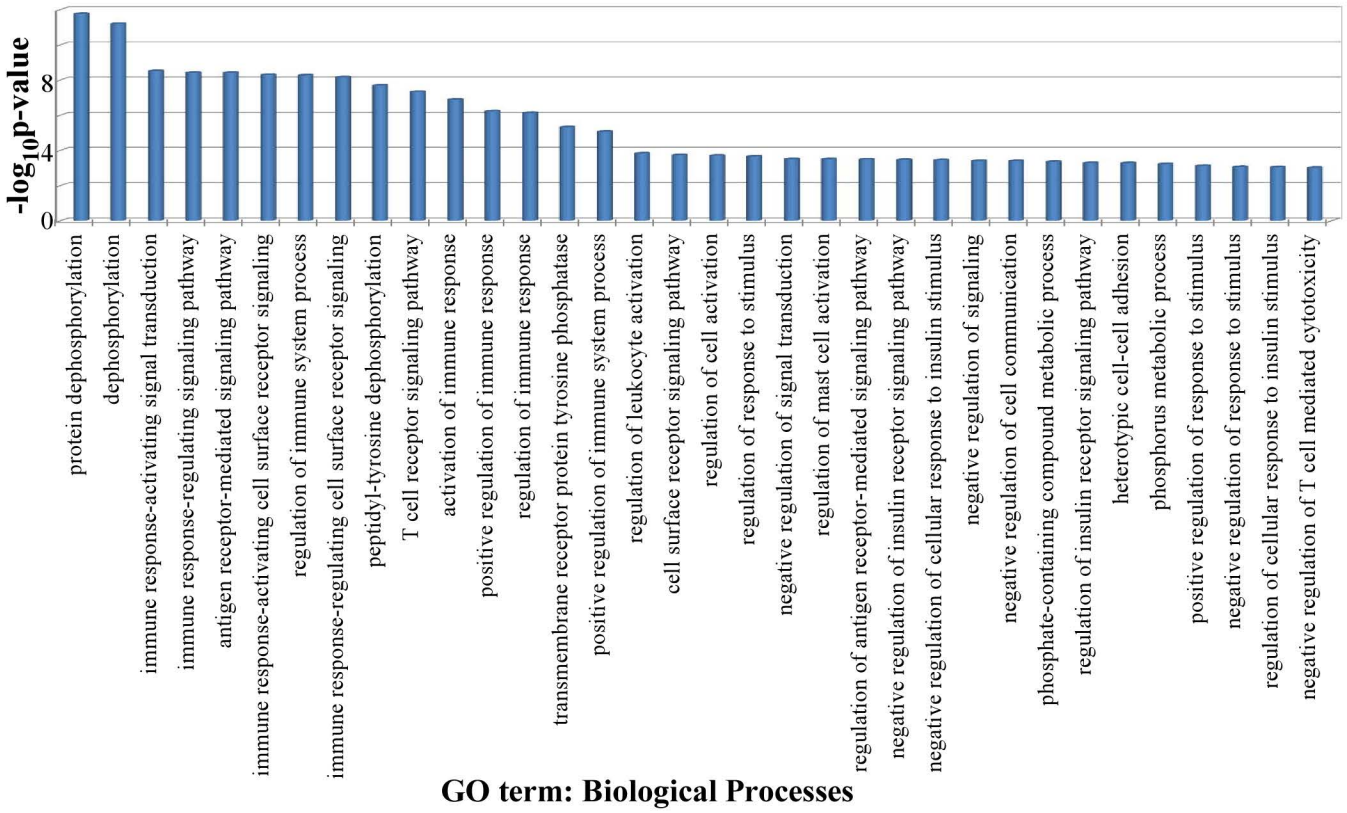
Metallophos
Group:
DSPP
Atypical PTP
Classical PTP
PSTPL

Figure 2

A.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4
8
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dephosphorylation
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regulation of immune system process
immune response-regulating cell surface receptor signaling
peptidyl-tyrosine dephosphorylation
T cell receptor signaling pathway
activation of immune response
positive regulation of immune response
regulation of immune response
transmembrane receptor protein tyrosine phosphatase
positive regulation of immune system process
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negative regulation of insulin receptor signaling pathway
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negative regulation of signaling
negative regulation of cell communication
phosphate-containing compound metabolic process
regulation of insulin receptor signaling pathway
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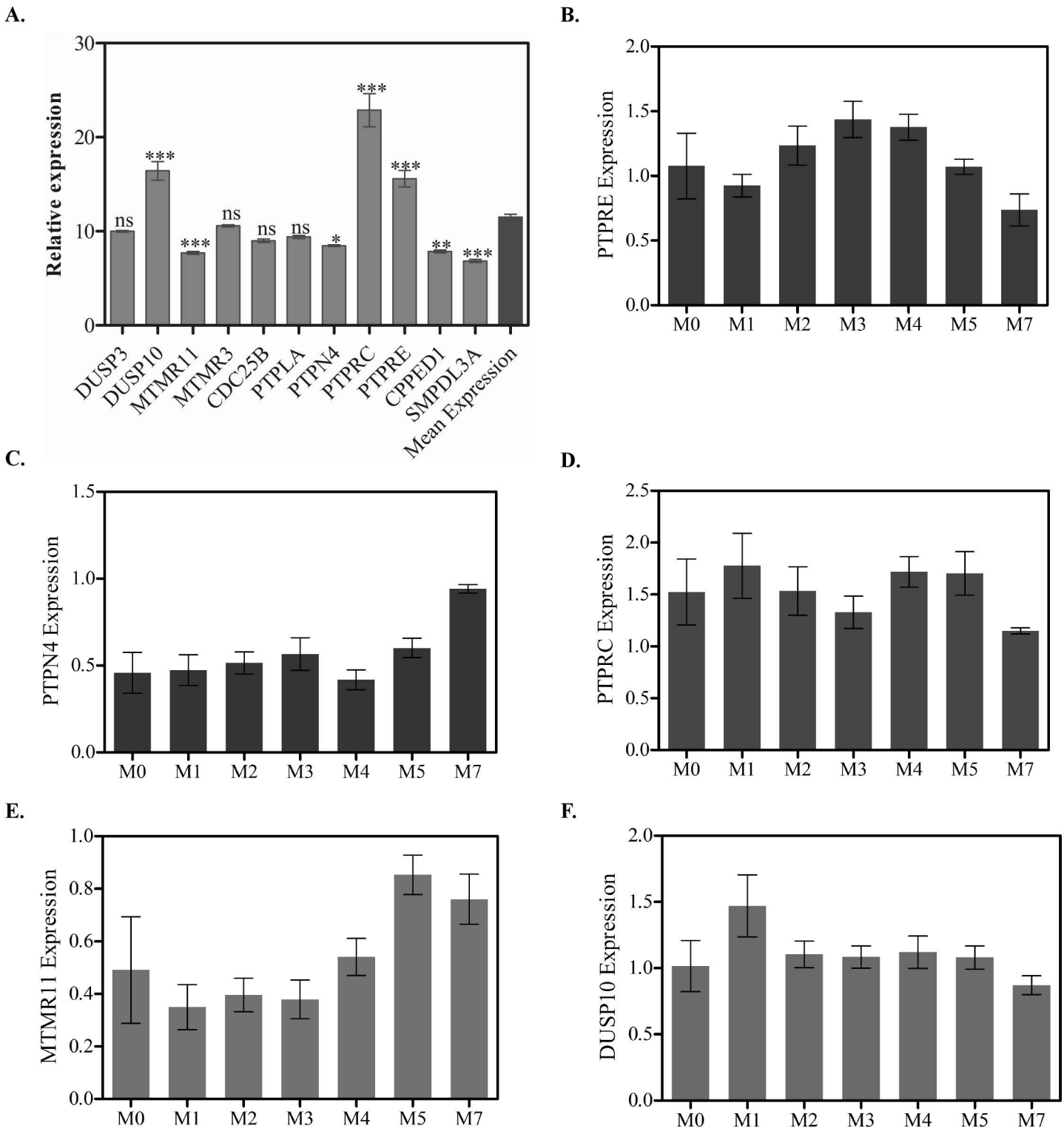
negative regulation of T cell mediated cytotoxicity
GO term: Biological Processes

Figure 3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DUSP10
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PTPLA
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PTPRE
CPPED1
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Mean Expression
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PTPRE Expression
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M2
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M5
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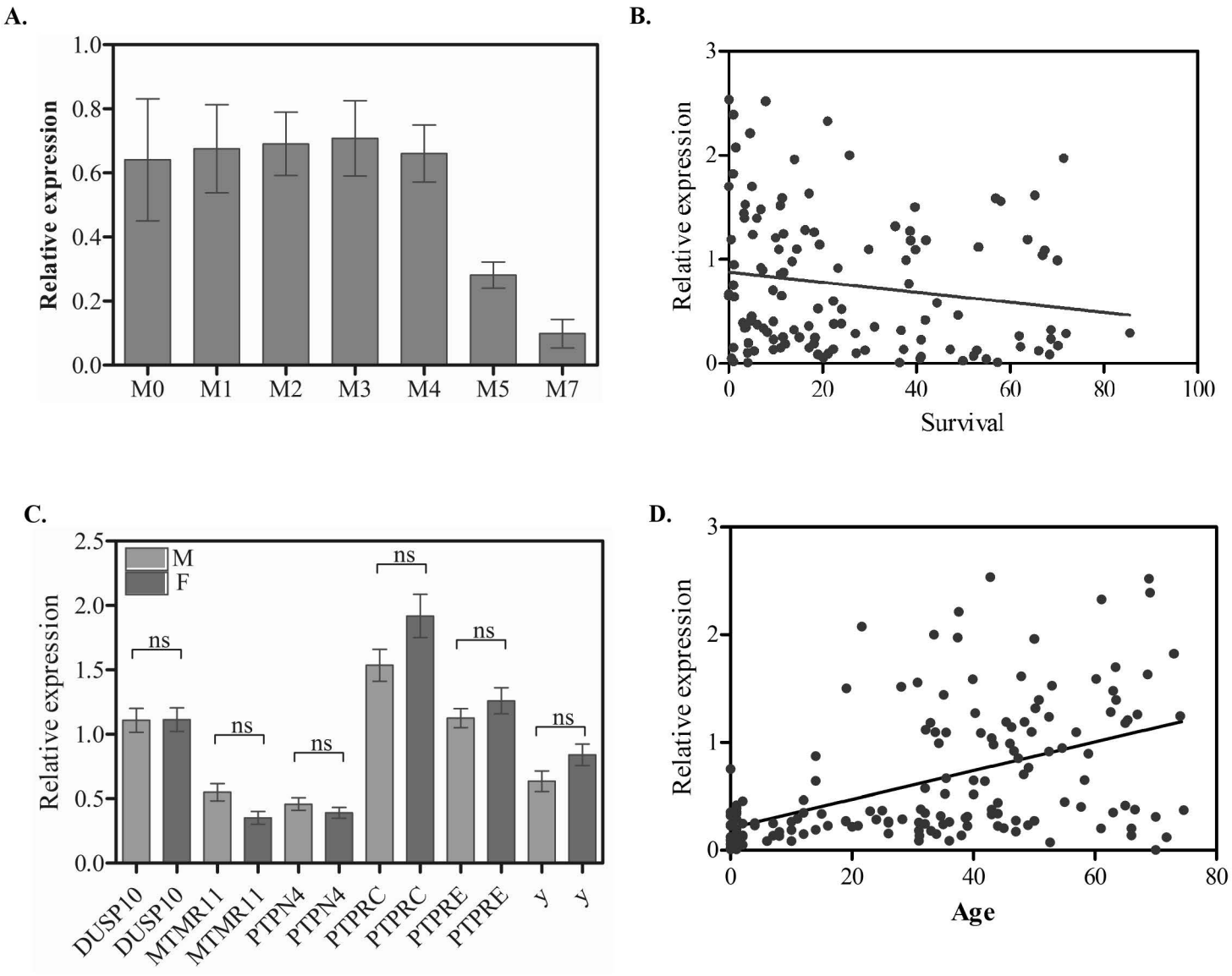
Figure 4


A.
Relative expression
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0.4
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M1
M2
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M4
M5
M7
B.
Relative expression
3
2
1
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0
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40
60
80
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Survival
C.
Relative expression
2.5
2.0
1.5
1.0
0.5
0.0
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F
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DUSP10
DUSP10
MTMR11
MTMR11
PTPN4
PTPN4
PTPRC
PTPRC
PTPRE
PTPRE
y
y
D.
Relative expression
3
2
1
0
0
20
40
60
80
Age

Figure 5