DPYSL3 is a multifunctional modulator in claudin-low breast cancer

Ryoichi Matsunumaa and Matthew J Ellis*

1 Lester and Sue Smith Breast Center, Baylor College of Medicine, Houston, USA
2 First Department of Surgery, Hamamatsu University School of Medicine, Hamamatsu, Japan
3 Hamamatsu Oncology Center, Japan

Commentary

Proteogenomics is the field of integrating data from mass spectrometry-based shotgun proteomics, and phosphoproteomics into next-generation RNA and DNA sequencing data analysis pipelines that promises new insights into cancer biology and therapeutic targeting. As well as analyses of clinical samples for disease phenotype association analysis, the application of proteogenomics to model systems also has considerable potential. Patient-derived xenografts (PDX) generated in immunosuppressed mice strains provide a useful setting to analyze the biological properties of the intrinsic subtypes of breast cancer because this approach effectively captures the biological diversity of this disease [1]. The Clinical Proteomic Tumor Analysis Consortium (CPTAC) generated quantitative iTRAQ mass spectrometry-based proteomics and phosphoproteomics data across the WHIM series of PDXs tumors that was combined with RNA and DNA sequencing information to provide integrated proteogenomic profiles [2]. Herein we explored these data to identify extreme outliers in the proteogenomic data that were Claudin-low (CLOW) subtype-specific and had not previously studied in breast cancer. WHIM12 breast cancer PDX was previously classified as a high confidence CLOW tumor based on transcriptomic profiling [2]. A CPTAC proteogenomic analysis prioritized dihydropyrimidinase-like-3 (DPYSL3) as a multi-level (RNA/Protein/Phosphoprotein) expression outlier specific to the CLOW subset of triple negative breast cancers. These data suggested high-levels of DPYSL3 expression and phosphorylation were associated with CLOW breast cancer and thus DPYSL3 may regulate some of the unique biological features of this subtype. In our view, discovery approaches that trangulate multiple tiers of ‘omics data with literature search engines to identify novel and targetable cancer biology should be more widely applied.

DPYSL3 is a phosphoprotein and a member of DPYS gene family that shares about 50-70 % sequence homology [3-6]. DPYSL3 is highly expressed in developing and adult nervous systems [6-8] and functions in a variety of cellular processes, including cell migration, differentiation, neurite extension, and axonal regeneration [9,10]. DPYSL3 regulates the actin and microtubule growth cone cytoskeleton [10,11,22,24], and other publications demonstrate GSK3 regulates mitotic chromosomal alignment through phosphorylation of DPYSL3 and mitotic progression through its effect on spindle microtubules [25]. In CLOW breast cancer, loss of DPYSL3 causes multiple nucleation and polyploidy during mitosis of CLOW cells was confirmed by studies in multiple cell lines. DPYSL3 interacts directly to tubulin, promotes microtubule assembly and is also known as a microtubule associated protein [22,23]. In fact, Immunoprecipitation and mass spectrometry data from CLOW cell line models demonstrates DPYSL3 interacts with tubulin β. In addition, there are some previous reports that demonstrate DPYSL3 regulates the actin and microtubule growth cone cytoskeleton [10,11,22,24], and other publications demonstrate GSK3 regulates mitotic chromosomal alignment through phosphorylation of DPYSL3 and mitotic progression through its effect on spindle microtubules [25]. In CLOW breast cancer, loss of DPYSL3 causes multiple nucleation and

*Correspondence to: Matthew J. Ellis, Lester and Sue Smith Breast Center, Baylor College of Medicine, Houston, Texas 77030, USA, Tel: (713) 798-1999; E-mail: matthew.ellis@bcm.edu
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then polyploidy due to cytokinetic failure likely due to disruption of vimentin function through increased phosphorylation. In other words DPYSL3 is a negative regulator of a vimentin kinase(s) which has yet to be identified.

In contrast, the role of DPYSL3 in migration and EMT in CLOW cells does appear to be PAK dependent and specifically mediated by PAK2. Cell migration in DPYSL3+ cells correlated with increased phosphorylation of PAK2 on Ser20 and was sensitive to PAK2 siRNA and pharmacological PAK inhibition. Immunoprecipitation and mass spectrometry-based proteomics or western blotting suggested that DPYSL3 and PAK2 directly interact such that DPYSL3 may function as a direct negative regulator of PAK2. Thus a PAK inhibitor could potentially mitigate increase migration as an adverse effect of DPYSL3 targeting. Importantly a PAK inhibitor does not differentially affect the viability of WHIM12 shLuc versus WHIM12 shDPYSL3 cells and a PAK inhibitor had no effect on a distribution of cell cycle in both of WHIM12 shLuc and shDPYSL3. Thus, DPYSL3 can be considered a biomarker for CLOW tumors where migration and EMT can be potentially resistant to a PAK inhibitor.

A role for DPYSL3 in EMT is consistent with Parsana's multi-study integration data [26]. Furthermore WHIM12 was derived from a patient with a metastatic carcinoma with spindle cell formation, an EMT characteristic. Further analyses of human data (METABRIC) in tumors designated CLOW subtype revealed a strong positive correlation between multiple EMT markers and DPYSL3 expression. This finding is consistent with induction of DPYSL3 by Snail and Twist in the HMLE EMT system in which HMLE-Snail and HMLE-Twist cells were generated by infecting immortalized human mammary epithelial cells with retroviral vectors expressing Snail or Twist. While it appears somewhat paradoxical that a negative regulator of EMT is positively associated with EMT markers in clinical data sets a logical synthesis of these data is that DPYSL3 is induced by EMT and subsequently provides negative feedback on the EMT state by inhibiting PAK. Within a tumor where EMT is the dominant and pathological state, DPYSL3 mediated feedback maybe impaired; causing DPYSL3 levels to increase yet EMT persists. DPYSL3 is induced by retroviral expression of Twist and Snail in this model, thus potential feedback inhibition in the transcription of Snail and Twist by DPYSL3 is disrupted because these EMT transcription factors are not controlled by their normal regulatory elements. Certainly the kinetics and exact mechanism of how DPYSL3 regulates EMT warrants further detailed study.

In conclusion, our study provides insights into DPYSL3 as a negative regulator of kinases that modulate cytokinesis, motility and EMT in CLOW breast cancer. DPYSL3 expression identifies CLOW tumors that will be sensitive to approaches that promote vimentin phosphorylation during mitosis and inhibitors of PAK signaling during migration and EMT. Therefore DPYSL3 expression could thus be used to stratify patients for clinical trials that target these cancer hallmarks.

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