Pan-cancer Analysis Predicts Kindlin-associated Global Mechanochemical Perturbation

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Pan-cancer Analysis Predicts Kindlin-associated Global
Mechanochemical Perturbation

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Abstract

Kindlins are mechanosensitive adapter proteins that connect extracellular mechanical cues to intracellular chemical events. Any alterations in these proteins thus alter cellular signaling, which could result in cancer progression. However, their involvement in global mechnanochemical signals remains elusive in cancers. Here we analyze pan-cancer samples to decipher how kindlin alterations aid cancer progression. We show that kindlin alterations, at both the genetic and mRNA level, dysregulates cellular behavior which significantly correlate with poor survival. We find that while these alterations are cancer-specific, they are prevalent in advanced tumor stages and metastatic onset. We observe that kindlins co-alter with a substantial fraction of human mechanochemical proteome in various tumors. Our analysis suggests how kindlin alterations aid tumor-promoting signals with a synergistic effect from alterations of cancer-hallmark genes. Notably, we demonstrate a consistent alteration of epithelial-mesenchymal-transition markers with kindlin activity. Overall, our study highlights how kindlin alterations could affect metabolism, genomic instability, and signal disruption via their interactome network, causing cancer and suggests targeting them as a therapeutic strategy.
Introduction

Cancer is a multiplex physiological disorder projected to be nearly 1.5 times deadlier in the coming decades. Severity of this disease originates from the rapid migration, protrusion, tissue invasiveness, metastatic capacity, and chemotherapy-resistant properties of malignant cells. These processes are constrained by many internal and external cues, including mechanical force transmitted from extracellular matrix (ECM) proteins. These Cell-ECM interactions are driven by a specialized transient structure called focal adhesion, which forms the mechanosensory hub by recruiting hundreds of force-sensing proteins to orchestrate intracellular rearrangement according to external mechanical cues. Due to the presence of these proteins, the site-specific mechanical cues are translated into chemical signals within cells, further rewiring the genetic and epigenetic landscape, favoring cancer growth. One such family of mechanosensing adapter proteins, the kindlins, convey the extracellular message of integrin outside-in signals via physical interactions to various structural proteins, receptors, and transcription factors, producing a plethora of chemical effects. These proteins are explicitly related to almost every cancer-hallmark protein, showing their importance in cancer onset, progression and recurrence. Kindlins are known to play important roles in signaling pathways corresponding to tumor-microenvironment manipulation, cellular metabolism, cell cycle progression, transcriptional regulation, and cancer stem cell regulation. Any alteration or structural mutations in these proteins might affect their mechanochemical signaling in a global scale leading to altered homeostasis.

Mutations in the regulatory region of a gene either up or downregulate its expression, while coding mutations can alter the proteins' stability, flexibility, and binding affinity for the respective ligands, thereby hindering signal transduction. However, the current data are insufficient to model the actual effect of mutations on the intrinsic properties, especially for the mechanosensitive proteins. The stochastic nature of tumor heterogeneity, due to the involvement of signal crosstalk and co-occurrent mutations in involved proteins, also makes the cancer systematics intricate and complicated to regard the effect of genomic and proteomic alterations. Moreover, the current way of tracing a high-impact alteration solely based on a statistical analysis of clinical data belies the structural and chemical consequences. Oversight of these aspects thus encumbers the coherent understanding of the intricate mechanisms of cancer progression.

In the present study, we have conducted a pan-cancer analysis of FERMT genes based on the clinical samples of International Cancer Genome Consortium (https://dcc.icgc.org), The Cancer Genome Atlas (https://www.cancer.gov/tcga), Catalogue of Somatic mutations in cancer (https://cancer.sanger.ac.uk/cosmic), and PCAWG study, utilizing genetic alterations, protein structural analysis tools, and gene ontology datasets to study the pan-cancer alteration effects of kindlin family proteins in mechanochemical signaling. While we show that kindlins are responsible for tumor progression, and the onset of metastasis, our analysis also reveals a prominent role of kindlins in epithelial-mesenchymal transition correlating to alterations in major mechanosensitive proteins. This study plausibly reveals an association of kindlin dysfunction with poor disease-free survival. Furthermore, using normal mode analysis (NMA), we have predicted the impact of cancer-specific mutations on the structural stability of kindlin proteins and how these mutations perturb their signal receiving and transducing ability, which in turn could regulate their downstream signal transduction pathways. Finally, this structural genomics approach successfully correlates the alteration effects to the clinical parameters substantiating the mechanochemical role of kindlins in different stages and subtypes of cancer.
Results

Kindlin alterations are found across multiple cancer types:

Using our protocol (Fig. S1), three types of kindlin family genes were found altered in 45 different cancer types (sample size, n=2922), with FERMT1 (kindlin1 gene) being the major contributor (8%) followed by FERMT2 (kindlin2 gene, 6%) and FERMT3 (kindlin3 gene, 5%) (Fig. S2) above a z-score cut-off ±1.96 (p-value < 0.05). Majority of these alterations are either mRNA expression or gene amplification (Fig: 1a). Alteration frequencies are high in the case of non-solid (e.g., leukemia, lymphoma) or soft-tissue cancers (e.g., leiomyosarcoma) (Fig: 1a). FERMT1 alterations are found in 21 types of cancers whereas FERMT2 is altered in 15 types (Fig. S2a and b).

Kindlin expression level associates with stiffness-induced breast-cancer invasiveness and metastasis\textsuperscript{24}. In our sample cohort, we found that both FERMT1 and FERMT3 mRNA are overexpressed in tumor samples. However, the increase in mRNA expression is not significant for FERMT3 when compared to FERMT1 (Fig. 1b). Interestingly, FERMT2 expression is significantly decreased in these tumors when compared to the normal samples (Fig. 1b, Fig. S3). Among blood cancers, expression of FERMT1 and FERMT2 is highest in T-cell lymphoma, unlike FERMT3 expression, which is lowest compared to other subtypes (Fig. S4-S6). Similarly, both FERMT1 and FERMT2 expression are higher in luminal breast cancer and MSS form of colon cancer (Fig. S4, and S5), while in gastric MSS, FERMT3 expression is higher (Fig. S6). FERMT1 expression increases in higher stages of cancer progression in BLCA, COAD, LUSC, and STAD (Fig. S7). Stage-specific decrease in FERMT2 mRNA expression is not pronounced except in renal cancer, LUAD, and BRCA (Fig. S7). The increase in FERMT3 mRNA expression shows significant stage-specific increase only in renal cancer and uveal melanoma (Fig. S7). These expression changes in kindlins are related to the overall mutational load in samples. With increasing mutations in cancer samples, FERMT1 and FERMT3 expression levels increase significantly, while FERMT2 shows an inverse trend ($p_{\text{FERMT1}}=4.184 \times 10^{-15}$, $p_{\text{FERMT2}}=1.356 \times 10^{-14}$, $p_{\text{FERMT3}}=6.687 \times 10^{-07}$), albeit not so strong correlation ($\rho_{\text{FERMT1}}$~0.25, $\rho_{\text{FERMT2}}$~ -0.25, $\rho_{\text{FERMT3}}$~0.16) (Fig. 1d). To check whether the mRNA expression is consistent to the protein abundance, we analyzed the CPTAC pan-cancer proteome data from TCGA samples by comparing tumor and tumor-adjacent normal tissue. In contrast to the mRNA expression level, FERMT1 protein expression decreases in maximum cancer samples. However, FERMT3 protein expression shows a mixed nature of up-and down-regulation in a cancer-specific manner (Fig. 1c). To find the plausible cause, we examined the expression level of the kindlin-associated miRNAs from cancer samples (Table S1-S3). These results not only indicate miRNA-mediated kindlin expression in cancer-specific manner but also suggest a feedback-like looping between kindlin expression and miRNA expression. This connection of kindlin/miRNA axis to cancer progression and chemoresistance is consistent with experimental evidences\textsuperscript{25,26}.

To investigate whether kindlin mRNA expression can correlate with cancer prognosis, we performed the expression-specific analysis of overall survival and disease-free survival (Fig. 1e-j). High expression of FERMT1 can be associated with poor overall survival (p < 0.001, hazard ratio (HR) = 1.9) (Fig. 1e), while overall survival appears to be independent of FERMT2 (Fig. 1f) and FERMT3 (Fig. 1g) expression. Interestingly, higher FERMT2 expression can be correlated with lower disease-free survival (Fig. 1i), unlike the other two FERMT genes (Fig 1h, 1j), suggesting its role in chemoresistance or cancer recurrence, as evidenced in a study by Ning et al.\textsuperscript{27} Individual cancer analyses show FERMT1 overexpression as a prognostic marker in PAAD (p=0.03, HR=1.6) and SKCM (p<0.001, HR=1.7) (Fig. S8). Underexpression of FERMT2 may correlate with lower survival in BLCA (p=0.0036, HR=1.6) and STAD.
Interestingly, FERMT3 overexpression is prognostic for LAML (p=0.001, HR=2.5), while under-expression is prognostic for SKCM (p=0.0019, HR=1.52) (Fig. S10). FERMT1 overexpression corresponds to poor disease-free survival (DFS) in PAAD (p=0.0019, HR=2) (Fig. S11), while the same in the case of FERMT2 in ACC (Logrank p=0.034, HR=2.1) and COAD is noticeable (p=0.033, HR=1.7) (Fig. S12). FERMT3 underexpression in CHOL (p=0.0066, HR=4.0) and overexpression in UVM (p=0.00067, HR=6.7) are strongly associated with poor DFS (Fig. S13).

Apart from expression, we analyzed the copy number variation (CNV) of FERMT genes in 33 types of cancer (Fig. S14). Except for LAML, THCA, and PRAD, other cancer types show significant CNV of FERMT genes in the form of heterozygous CNV (Fig. S14a). FERMT1 and FERMT3 mostly show heterozygous amplification across cancer types as opposed to FERMT2, which shows mostly heterozygous deletion (Fig. S14b). DNA methylation, another key regulator of Kindlin gene expression, is also significantly altered in 14 cancer types (Fig. S15). FERMT2 hypermethylation is significant in tumors showing the most prominent effect in KIRP. Although FERMT1 is hypomethylated in most tumor types, it is significantly hypermethylated in BRCA. Independent of hyper- or hypo-methylation, changes in methylation amount in kindlin genes anti-correlates with gene expression suggesting a downregulation most saliently for FERMT3 (Fig. S15b). FERMT1 and FERMT2 hypermethylation are markers of survival risk in LGG (Logrank p < 10^{-10}), ACC (Logrank p < 10^{-5}) and KIRC (Logrank p < 0.05), SARC (Logrank p < 0.05) respectively.

FERMT mutations are linked with tumor progression and metastasis:

FERMT mutations are found in 31 cancer types with mutation frequencies of 15%, 14%, and 12% for FERMT1, FERMT3, and FERMT3, respectively (Fig 2a). Cancer-specific coding somatic mutations are mostly missense mutations, followed by silent and frameshift insertion or deletion (Indel) mutations (Fig. S16). These mutations are distributed throughout their sequences (Fig 2b). However, the mutation frequency within the FERM domain of FERMT3 is considerably high. In the case of FERMT2, we observe a mutational hotspot partially within the F1 domain. We have mapped the potential impact of mutations in the regulatory region on mRNA expression through the extent of loss of function, gain of function, or change of function from wild-type functionality (inferred from recurrence and multiplicity in tumor samples28 (Fig. 2c). Across FERMT1 and FERMT2, most of the high-impact mutations originate from the 5'UTR and upstream sequence. Because of these regulatory mutations, the expression of FERMT1 and FERMT2 has decreased consistently, whereas it increases for FERMT3. In addition, the impact of regulatory upstream or downstream mutations across FERMT3 has remained low contrasting to some start-loss, stop-loss, stop-gained, splice-region and intronic mutants which remain high-impact. The Kaplan-Meier curve shows a significant risk of survival conferred by FERMT mutations (p-value = 0.0003, HR = 1.932) (Fig. 2d). Upon further analysis, we found that FERMT1 and FERMT3 mutations have almost the same survival risk, and are higher compared to the FERMT2 mutation-associated risk (Kruskal-Wallis rank sum p-value: FERMT1-FERMT2, 0.0058; FERMT2-FERMT3, 0.0002; FERMT1-FERMT3, 0.2945) (Fig. 2e). Tumor stage-specific mutation analysis shows an almost similar trend for all kindlins, as most of these mutations are found at tumor stages T2 and T3, indicating their effect on tumor progression rather than onset (Fig. 2f). FERMT mutations are also significant in the metastatic M0 stage compared to later stages, suggesting their role before metastatic onset (Fig. 2g).

Mutations affects structure-function dynamics of kindlins:
Kindlin family proteins show ~49-58% sequence identity and ~67-73% sequence similarity, but they are structurally similar (Fig. S17). To study the effects of mutations on structural stability of kindlins, we calculated the ΔΔG values of all the cancer-specific mutated conformations of all kindlin types. Since the mechanochemical activity of kindlins comes from their domain-specific flexibility, we calculated the vibrational entropy change (ΔΔS) of mutants concerning the wild type versions. Our analysis reveals four different populations of these mutants: both high flexibility and stability (Q1), low flexibility and high stability (Q2), both low flexibility and stability (Q3), and high flexibility and low stability (Q4) (Fig. 3a-c). We also get a trend towards decreasing stability with increasing flexibility (p<2.2e-16; σFERMT1=-0.7470601; σFERMT2=-0.8190608; σFERMT3=-0.7077385). Furthermore, we classified the mutants into five categories: very high, high, moderate, low, and slight for each stabilizing and destabilizing cohort (Table S4-S6). Almost 50% cancer-specific mutants of all kindlins are loss-of-function and highly disease causing, while the rest ~50% are tolerable in cells (Fig. 3d). Most of the loss of function mutants are from both very high stability/low flexibility and very high flexibility/very low stability region i.e., Q2 and Q4, respectively. ΔΔG analysis of respective dimers also shows a common destabilizing effect for all the mutant dimers (Fig. 3e-3g). Mutation-induced protein structural alterations can lead to changes in chemical properties due to altered intramolecular interactions (Fig. S18-S23). Our analysis predicts pronounced changes in hydrophobicity in mutant monomers and dimers of all kindlins (Fig. S24, S25). However, no significant changes in intramolecular salt bridge interactions are seen considering minimally altered κ-values, a parameter indicating extent of intramolecular salt-bridge formation (Fig. S26, S27).

Phosphorylation is another important aspect of kindlin functionality, which has been validated experimentally at T8 and T30 position for kindlin1; Y193, S159, S181, and S666 for kindlin2; T482 and S484 for kindlin3. Computational predictions indicate a complete loss of T8 and S484 mutation sites in kindlin1 and kindlin3, respectively. For FERMT2, all frameshift mutants show a complete loss of Y193 and S666 phosphorylation sites (Table S7-S9). These structural effects on phosphorylation correlate with patient-specific phosphorylation level. From phosphoproteomic tandem mass tag (TMT) data, an overall decrease in phosphorylation is observed for all three kindlins unlike FERMT2, which shows that elevated phosphorylation levels (Fig. S28) might be due to altered phosphorylation sites. Overall, this affected phosphorylation might be attributed to the altered kinase activity of ser-thr/ tyr kinases, a signature of tumor cells.

With all kindlins, insertion-deletion (in-del) frameshift mutations lead to the loss of one or more biologically active domains in the protein (Fig. 4). Most of the FERMT3 in-del mutants except D231del, retain all domains with either altered sequence or altered three-dimensional domain structure. Similarly, K154del kindlin2 mutant retains all domains as wild type but with distorted dimerization domain, FERM and PH domain, indicating a loss of important functionalities (Fig. 4b and d inset).

**Kindlin mutations cause alterations in mechanochemical signal transmission:**

Kindlins are known to carry out their mechanochemical signal transduction by their domain-specific movement (Fig. 5a). This mechanotransduction and its perturbations can be measured by normal mode analysis (NMA). We have performed NMA of the mutant and the wild-type kindlins in the presence of a computationally predicted force range of 87 pN. Previously, it was shown that force transmitted to kindlins through integrin outside-in signaling strengthens the dimerization of kindlins reinforcing integrin condensation at focal adhesion sites. Our analysis reveals force-induced movements of three specific regions- the F0 domain that interacts with the F-actins and paxillin; the PH domain that connects plasma membrane via...
PIP2 and might respond to membrane-mediated signaling events\textsuperscript{34,35}; and F3 domain that directly binds to integrin, TGFβRI, and RTKs through F3 domain\textsuperscript{36,37} (Fig 5b). B-factor analysis of force-induced root mean squared fluctuations (RMSF) predicts any changes in atomic fluctuation of cancer-specific mutants in all three kindlins (Fig. S29), with significant changes in the very highly stabilizing/destabilizing and multiple mutants, as might be expected.

This change is evident for kindlin1 and kindlin2 in their dimerization domain, PH, and F3 domain (Fig. 5b). In the W65G mutant, the kindlin2 dimerization region shows a high RMSF, plausibly destabilizing dimerization, while C309S loses its flexibility in the F-actin/paxillin binding region (Fig. 5b). The multiple mutant R255C-R526K-I1160T variant and the R526K-I300V variant of kindlin1 appear to lose the flexibility of the F3 domain, indicating a loss of the spring-like force-transmitting property (Fig. 5b). F3 mutants show minimal changes in domain-specific RMSF (Fig. 5b). The changes in the dimerization properties of the mutants are also evident from the prediction of the dimerization affinity (Fig. 6a-6c). Kindlin2 completely loses its dimerization affinity in all mutants (Fig. 6b). In contrast, kindlin3 mutants appear more stably dimerized (Fig. 6c), while kindlin1 mutants show a mixed effect on the dimerization trait (Fig. 6a) suggesting altered kindlin-dimer functionalities.

We further analyzed the signaling properties of kindlins and their changes in kindlin mutants using the Markov chain model\textsuperscript{38} (Fig. S30-S41). In the case of kindlin1, the receiving signal rate was sporadically perturbed across the sequence for multiple mutations, whereas transmitter signals were significantly unaffected. For single missense mutations, the recipient signaling rate is slightly perturbed within the 200-500 residue, along with trends in the 1-150 range for a tryptophan-removing mutation. However, the broadcasting signal rate is largely undisturbed in kindlin1 mutants. For Kindlin2 containing multiple mutations, there is some degree of interference across all FERM domains with receiver signaling rates. The broadcasting signal rate is not disturbed as much. Both receiving and transmitting signal rates are sporadically and significantly disrupted across all FERM domains for multiple mutations of Kindlin3 (Fig. S38-S41). As for Kindlin3 single mutants, the broadcaster rate appears to be altered in the F0, F1 and parts of the F2 domains, whereas the receiver signal affects the 200-450 amino acid index more significantly.

**Kindlin alterations are associated with global mechanochemical signal perturbations favoring cancer progression:**

The change in signal-receiving or signal-broadcasting properties of the mutant proteins might affect their ability to convert mechanical signals into biochemical cues, causing altered physical interactions. Our predictions of binding affinity change indicate altered kindlin-partner interactions in cancer-specific mutations. While kindlin1 mutants appear responsible for minor changes in homeostatic interactions, kindlin2 and kindlin3 cause a considerable decrease (Fig. S42). Most of these changes are either mutation-specific or interactor specific, or both. For kindlin1, its interaction with ILK is most affected among the mutants, except for two multiple mutants R255C-I1160T and Q26K-R17S-H38N, where the interactions increase (Fig. 6d). Depending on highly-destabilizing kindlin2 mutations, we found a 0.2-fold decrease in affinity for PFKM and an increased affinity of highly stabilizing and destabilizing mutants to TGFBR1 and FERMT3 (0.2-fold 0.1-fold respectively) (Fig. 6e). Kindlin3 interactions are slightly disturbed and show a trend toward decreasing affinity. However, in two Kindlin3 multiple mutants, V558F-A539S and V100I-L46F, their interactions with ILK, LIMS1, LSM8, and PARVA decrease sharply (Fig. 6f).

Kindlins subtypes form huge interacting networks due to their function as adapters (Fig. S43-S45) linking many major biological processes (Fig. S46). Mutations can alter their homeostatic interactions with wild-type interactors, while the interaction partners might also be altered in
cancer samples triggering a synergistic effect. Therefore, we checked the co-alterations of the corresponding physical interactors in cancer. In FERMT1 altered samples, TTC37, SKIV2L, and FERMT2 show maximum alterations compared to unaltered samples (Fig. 7a). Similarly, in FERMT2 altered cohort, its partners TGFBBR1, SEPTIN9, SEPTIN11, PXN, and PFKM are mostly co-altered (Fig. 7b). FERMT3 co-alters to almost the same extent as all its interactors (Fig. 7c). Notably, this also demonstrates a strong mutual exclusiveness (tendency towards co-occurrence) in alterations of all the kindlins in cancer (p-value <0.001, q-value <0.001, log2 odds ratio: FERMT1-FERMT2 >3, FERMT2-FERMT3 =2.77, FERMT1-FERMT3 =1.93).

Kindlins alterations accompany significant alterations in major cancer hallmark genes (Fig. 7d). For a better understanding of the synergistic effects via co-alteration analysis, we introduce a term ‘co-alteration dynamics’ of a biological process which denotes the average effect of co-alterations of the components of that particular biological process with respect to one or more components. For example, we calculated co-alterations of cancer-hallmark gene set with respect to kindlins, signifying co-alteration dynamics of cancer-hallmarks with respect to kindlins. Our co-alteration analysis containing 39 hallmark genes shows the most prominent co-alterations with FERMT2 (mean co-alteration dynamics=14.98) followed by FERMT1 (mean co-alteration dynamics=11.08), FERMT3 being the lowest (mean co-alteration dynamics=5.35). Kindlins act as interlinks of major cellular pathways, including other mechanosensitive or mechanochemical proteins directly linked either to kindlins or through kindlin interactors. Our meta-analysis revealed 62 proteins encompassing mechanochemical transcription factors, receptors, ion channels, cytoskeletal proteins, and other various types (Table S10). Almost all of these mechanochemical proteins are co-altered with all the Kindlins (Fig. 7e). Kindlin alterations mostly co-occur with ACTN1, ADGRs, DNMT1, RAC1, TMX4, and TP53. Here also, mechanochemical protein forming genes show the most prominent co-alterations with FERMT2 (mean co-alteration dynamics=19.3) followed by FERMT1 (mean co-alteration dynamics=12.34) and least co-alteration with FERMT3 (mean co-alteration dynamics=9.92). Interestingly, most of these co-altered mechanochemical proteins are either transcription factors or cytoskeletal proteins. To link these kindlin-associated co-alterations to cancer progression, we analyzed the contribution of kindlins in ten major cancer-associated pathways. FERMT1 alterations correlate with inhibitory effects on these pathways, whereas FERMT2 and FERMT3 alterations activate cancer-signature pathways of concern more frequently (Fig. 7f). However, FERMT1 alterations correlate with inhibition of apoptosis, cell cycle progression, DNA damage response, and androgen-receptor pathway like FERMT2 but to a lower extent (Fig. 7f). Interestingly, FERMT3 and FERMT2 alterations are significant in epithelial-mesenchymal transition (EMT). EMT-specific pathway enrichment analysis reveals a strong correlation between EMT-promoting processes like UV response downregulation, TGFβ signaling, angiogenesis, and hedgehog signaling to their alterations in 33 cancer types (Fig. S47). In parallel, EMT-inhibiting pathways like DNA repair, oxidative phosphorylation, and P53 tumor suppression are strongly anti-correlated with kindlin alterations (Fig. S47). FERMT2 alterations are also consistent with EMT-related immune filtrations supporting their significance in EMT (Fig. S48). While, FERMT2 correlates with the inhibition of apoptosis, we find consistent FERMT3 alterations with the activation of apoptotic pathways in cancer samples (Fig. 7f). Kindlins also correlate with other hormonal pathways; PI3K/AKT, mTOR, RTK, and MAPK pathways which are significant for any type of cancer progression.

Discussion

Clinical advancements in precision oncology currently face two major problems: understanding the tumor heterogeneity and predicting the trend in the intracellular complexity arising from modulated microenvironment. Mechanosensitive adapter proteins play an
indispensable role in these events linking extracellular mechano-environment with intracellular events through molecular clutch dynamics. Thus, alterations of these proteins could become the Achilles’ heel for cellular homeostasis, favoring malignancy. In fact, intra- and extracellular mechanical events are found to guide cancer progression, metastasis, and recurrence. Therefore, analysing the role of kindlins as one of the mechanosensitive adapters involved in diverse biochemical signaling and other regulatory functions might answer the mechanisms of cancer complexity and heterogeneity.

Previously, in the case of PAAD, it was found experimentally that kindlin downregulation contributes to intra-tumoral heterogeneity. Based on the nature of the kindlin distribution in normal tissues, we observe genomic alterations in cancers originating from different tissues. This leads us to propose a plausible role for changes in the kindlin family genes in contributing to regulating tumor heterogeneity. This heterogeneity corresponds to activation of different cellular properties within tumor cells. We have shown that kindlin-mediated biochemical alterations arise due to combined alterations of kindlins and their networks. Kindlin-mediated cancer-specific upregulation or downregulation of miRNAs can also be important for inducing malignancy and metastasis. Our analysis suggests an interesting feedback-loop mechanism of kindlin and miRNA expression, which has also been shown in breast cancer malignancies. We observed that miRNAs, regulated by kindlin2, target FERMT1 or FERMT3 mRNA. Another interesting observation is the correlation between total mutation and kindlin1, kindlin3 expression levels along with an anti-correlation between increase in genomic mutation and kindlin-2 expression. This advocates in favour of kindlin-mediated regulation of genomic instability, as was found experimentally by Zhao et al. for breast cancer. This suggests that kindlins activities are dependent on a balance of stability and flexibility. When this balance is perturbed, it will lead to disconcerted mechano-chemical signal transmission.

Kindlin dimerization is a key factor for integrin activation and mechanosensing. In the presence of ITG1BP1, kindlin-integrin interaction is competitively hindered and hence, the focal adhesion loses its stability. We found a trend of kindlin-dimerization disruption for mutated kindlin2. Moreover, ITG1BP1-Kindlin2 co-alteration is also anti-correlated (p-value <0.05, q-value <0.05, percent-altered in Kindlin2 altered cohort (n=195) ~1%, percent-unaltered in Kindlin2 altered cohort (n=2588) (~8%) which might ease focal adhesion disruption and hence aid cancer cell migration. All the other important focal adhesion proteins like VCL, PXN, ITGB1, and LSM8 are also significantly altered (Fig. 7a-7c) showing a prominent cancer-specific disturbance in the cell’s kindlin-associated mechanochemical hub.

Kindlin interactions are regulated in cancer either by expression or by kindlin mutations. Both mutation specific binding affinity and co-alteration of these direct interactors point to a global perturbation in signaling modules and their specific effects relating to wrong input of mechanosensing via kindlins. We analysed the functional involvements of kindlin-interactors from data available in Genecards (www.genecards.org) to get plausible altered roles of kindlins in cancer. Kindlin-1 direct-interactor TTC37 and SKIV2L are involved in protein folding due to their chaperone activity along with their role in super-killer complex. Both of them are significantly altered in cancer, suggesting a plausible path of kindlin-mediated protein folding and mRNA regulation alteration. PARVA, an important protein in sarcomere organization is co-altered significantly with all the three kindlins. Moreover, mutation induced altered interaction also suggests perturbed kindlin-mediated PARVA action in cancer. ILK and PARVB are known to form a complex to regulate cell motility, epithelial polarity, and the TNF pathway. Kindlins plausibly interacts with either ILK (Kindlin1/2/3) or PARVB (kindlin-3) to regulate this complex. Therefore, the altered interaction would be responsible for significant change in the activity of this complex in cancer cells. Kindlin2 is proven to be a key player in
TGBRI-SMAD3 interaction through direct binding\textsuperscript{48}, both of which are upregulated in cancer. Interaction analysis shows a higher affinity of kindlin2 mutants for TGBFRI and SMAD3, plausibly promoting TGFβ-signaling in kindlin2-mutant cancer-patients. Another kindlin2 interactor SEPTIN11 is also associated with chronic neutrophilic leukaemia. Due to mutations, a decreased possibility of kindlin2-SEPTIN11 interaction and further co-alteration in pan-cancer cohort has been observed. We see a significant co-alteration of kindlin-2 interactor MAPK, which was previously linked to chromosomal/microsatellite instability through COAD, GBM, and PAAD signaling pathways. Another cancer signature, telomere instability has been recently proved to be dependent on ECM stiffness and mechanosensitivity\textsuperscript{49}. Interestingly, kindlin3 interactors EXOSC10 and Kindlin1 interactor SKIV2L are linked to telomere maintenance. Alterations of these two proteins in cancer might therefore associate kindlin-mechanochemical signaling with cancer-specific telomeric instability.

Kindlins are also important for connecting mechanical environment to cellular metabolism. Previous reports suggest involvement of kindlin2 in amino acid biosynthesis and HIF-1α induced tumor growth in hypoxic condition\textsuperscript{16,50}. Our analysis suggests, a role of kindlin2 in GSK3 mediated inhibition of TCA cycle either via AKT1 or via CDC42 (Fig. S49). Predicted kindlin2 interactor PFKM is important in upregulating glycolysis. Although, specific role of this interaction is unknown, we can see a probable decrease in kindlin2-PFKM interactions in cancer. From this, we might hypothesize a PFKM-inhibiting role of kindlin2, which is altered in cancer thereby increasing glycolysis. This downregulation of TCA cycle and higher anaerobic glycolysis is a signature of cancer cell proliferation, growth, and chemo-resistance\textsuperscript{51}. In pathway-specific alteration analysis, we see a combined effect of all the kindlins, especially kindlin2 and 3, in activation of EMT accompanied by inhibition of DNA damage response. In addition, we see a role of kindlins in cell-cycle arrest, a common signature during EMT associated to increased ribosome biogenesis\textsuperscript{52}. Notably, we observe that kindlin1 alterations are correlated with negative regulation of cell-cycle progression, DNA-damage response, apoptosis as well as EMT. This might be because of patient-specific role rather than an overall scenario. A second possibility indicates its role in maintaining the temporal regulation of drug efflux, invasion, proliferation and stemness in different time points of EMT\textsuperscript{53}. Therefore, it becomes important to further elucidate the spatiotemporal role of kindlins in cancer progression and metastasis.

In summary, this analysis represents a pan-cancer scenario of how mechanosensitive adaptor-proteins like kindlins connect environmental mechanical cues and translate them to intracellular chemical events to favour cancer. This alteration seems an effect of altered intrinsic structural properties of kindlins – stability, flexibility, hydrophobicity, signal transmission property, and chemical properties – specific interactions, phosphorylation, and loss of functional domains, among others. A majority of these observations suggests correlations and not causality, hence demand further experimental mechanistic validations using structural, biochemical, and cellular approaches to find the exact causations in mechanistic details. However, it is certain that kindlins act as ‘super adaptors’ rendering many homeostatic and pathophysiological pathways and therefore, can be a good target for therapeutic strategy against cancer.

**Methods**

*Dataset Curation*
Preliminary genomic data for FERMT1, FERMT2, and FERMT3 was acquired from The Cancer Genome Atlas’ (TCGA, https://www.cancer.gov/tcga), pan-cancer analysis of whole genomes (PCAWG) study and processed. Cancer-associated somatic mutations were obtained from the Catalogue of Somatic Mutations in Cancer (COSMIC), and a dataset of multiple mutations per donor was curated from the International Cancer Genome Consortium (ICGC) database.

Analysis of Expression Levels

Donor-specific mRNA expression levels were obtained from the ICGC Data Portal for donors who possessed sequencing-based gene expression and array-based gene expression data. In addition to these donors across the Kindlins family (n=1045), mutation-specific expression data for genomic alterations (n=268; 18 cancer cohorts) in the non-coding regions was also obtained from the ICGC dataset. For both analyses, initial data was filtered for donors who possessed high and low functional impact mutations only, leaving out donors with mutations of unknown impact.

Furthermore, expression analysis across 33 cancer cohorts matching TCGA normal and GTEx data (https://gtexportal.org/home/) was performed using data from GEPIA, employing the one-way analysis of variance (ANOVA) test along with a q-value cut-off of 0.01 and a \( \log_2(TPM + 1) \) scale (TPM, Transcripts per million). Lastly, mRNA expression levels were analysed as a function of two clinical attributes – Tumor Stage (AJCC) and Metastasis Stage Code (AJCC) – using data from the PCAWG study.

For all genes outlined as part of the direct and indirect physical interactome of FERMT1, FERMT2, and FERMT3 (using BioGRID, https://thebiogrid.org/), we performed co-alteration of expression analysis on the PCAWG data to characterise their impact and predict their involvement in associated pathways and downstream functions.

For cancer and non-cancer patients, protein expression data was obtained and analysed using the Genomic Data Commons’ (GDC) Clinical Proteomic Tumor Analysis Consortium (CPTAC, NC/NIH) database that incorporates the TARGET (Therapeutically Applicable Research to Generate Effective Therapies) and TCGA libraries on cProSite (https://cprosite.ccr.cancer.gov/). Consequently, relevant microRNAs and their expression was analysed in the context of cancer via systematic analysis of primary literature and text-mining of high-throughput experimental data on miRDB (http://www.mirdb.org/) and miRCancer (http://mircancer.ecu.edu/).

Protein expression data analysis

Pan-cancer protein expression (mass-spectrometry) data were obtained from CPTAC dataset for 11 types of cancer. We obtained total 1272 tumor sample data and 808 tumor-adjacent tissue samples. Stomach cancer data was omitted due to no record of Kindlin expression in tumor adjacent samples. Protein expression data were collected as relative protein abundance in TMTlog2 ratio (TMT, Tandem mass tag) Patient-specific protein-phosphorylation data were collected from the same dataset. Phosphorylation analysis was available for 1272 tumor samples and 782 tumor adjacent samples. Phosphorylation alteration was calculated as:

\[
\text{Log2 Fold change} = \frac{\text{phosphorylation level (tumor)}}{\text{phosphorylation level (tumor adjacent tissue)}}
\]
Survival Analysis

Kaplan Meier (KM) plots for expression-specific overall and disease-free survival were performed using curated data originally taken from TCGA/ICGC cohort using cBioportal (https://www.cbioportal.org/) and GEPIA2 (http://gepia2.cancer-pku.cn/). Across 33 different cancer types. Overlapped samples (n=193) were excluded from the analysis. High mRNA expression data for which we have obtained were: 2405 (FERMT1), 2375 (FERMT2), and 2376 (FERMT3). Similarly low mRNA expression data were available for 2380 (FERMT1), 2376 (FERMT2), and 2376 (FERMT3) samples. Data from the PCAWG study and TCGA data (n=2583) for which status was available, was employed to curate mutation status-specific overall survival KM plots.

Mutated variant analysis:

COSMIC data were first collected individually for FERMT1, FERMT2, and FERMT3. Kindlin Point mutated samples were collected from curated dataset containing PCAWG, TCGA, ICGC data. Overlapped samples were filtered out which obtained a total 981 samples. Based on consensus transcript sequences for FERMT1, FERMT2, and FERMT3, multiple mutations per transcript (having same transcript ID within individual sample ID) were also recorded (n=38). These multiple-nucleotide variants (MNV) were further assessed and filtered manually applying MNV calling criteria by Wang et al. Functional impact i.e., loss-of-function or neutral, of the single nucleotide mutations were obtained using SIFT.

Cancer-Specific Mutational Stability Characterisation

The monomeric structures for FERMT1 (Q9BQL6), FERMT2 (Q96AC1), and FERMT3 (Q86UX7) were derived from AlphaFold. Dimers for each of them were prepared by performing homology modelling on trRosetta. Structural homology was further checked using POSA. Cancer-specific substitution mutations and frameshift insertion-deletion mutations were incorporated using mutagenesis tool of PyMOL (v. 2.5.2, The PyMOL Molecular Graphics System, Version 1.2r3pre, Schrödinger, LLC) and trRosetta, respectively.

Perturbations in the dynamics and stability of FERMT1, FERMT2, and FERMT3 dimers as a function of cancer-specific mutations were characterized employing an Elastic Network Contact Model–based normal mode analysis (ENCoM–based coarse-grained NMA) to compute predicted changes in folding free energy (ΔΔG) and change in vibrational entropy (ΔΔS). The ENCoM–based NMA approach takes into consideration the distinct nature and consequent effects of specific amino acids on the dynamics of the structure. Moreover, the involvement of vibrational normal modes and entropic analysis within the NMA method represents a novel approach to characterising protein structure dynamics and the effect of mutations.

Preparation of Insertion-deletion Mutant Structures

FASTA protein sequences for FERMT1, FERMT2, and FERMT3 were obtained from NCBI following the correct amino acid sequence length within the Homo sapiens collection of transcripts. Each of the sequences were queried against translated nucleotide databases using the TBLASTN tool’s BLOSUM62 matrix adjusted for conditional compositional score, and existence:11 extension:1 gap cost. Resulting sequences producing significant alignments were
sorted by highest percentage identity with their respective query sequences. With the three best matches against FERMT1, FERMT2, and FERMT3, insertion-deletion mutations obtained from COSMIC\textsuperscript{54} were incorporated within each nucleic acid sequence. These mutated sequences were translated to their respective peptide sequences using EMBOSS Transeq. For each nucleic acid sequence, we employed frame 1, and standard code parameters. The protein sequences obtained were screened and submitted on the trRosetta server\textsuperscript{61} to predict insertion-deletion mutant structures. The structures with the highest homology modelling score were selected for downstream analysis.

**Structural Analysis of Cancer-specific Variants**

The characteristic deviation of stability ($\Delta \Delta G$) and flexibility ($\Delta \Delta S$) of cancer-specific mutant variants with reference to the wild type structures was performed using the ENCoM – based NMA method\textsuperscript{63}, allowing for a more accurate representation of intramolecular interactions and the prediction of consequent effects on conformational stability and molecular flexibility\textsuperscript{18}.

In our analysis $\Delta \Delta G$ was represented as:

$$\Delta \Delta G = \Delta G(\text{wild-type}) - \Delta G(\text{mutant})$$

And thus, $\Delta \Delta G = (+)$ ve means stabilization and $\Delta \Delta G = (-)$ ve means destabilization; $\Delta G$, free energy change.

Similarly, $\Delta \Delta S$ was calculated as:

$$\Delta \Delta S = \Delta S(\text{wild-type}) - \Delta S(\text{mutant})$$

Here, $\Delta \Delta S = (+)$ ve means increased flexibility and $\Delta \Delta S = (-)$ ve means decreased flexibility. $\Delta S$, change in vibrational entropy.

Preliminary data collected from all cancer-associated somatic mutations via this method was then filtered to screen for mutations that corresponded to a $\Delta \Delta G_{\text{ENCoM}}$ value of $\geq +1.24$ and $\leq -1.24$ for further analysis (as very highly stabilizing and destabilizing mutants).

Furthermore, the B-factor (generally termed as the Debye-Waller Factor) was computed using the following formula to predict the flexibility of each amino acid residue within the respective chains, where $\Delta R^2$ indicates the oscillation of every residue from its equilibrium position\textsuperscript{64}.

$$B\text{ factor} = \frac{8}{3} \pi^2 < \Delta R^2 >$$

Following NMA, the deformation energies and eigenvalues of multiple low-frequency normal modes were computed using the below-mentioned formula for all wild type and mutant variants to measure the degree of collectivity for each mode, and consequently predict the extent of displacement of large regions of the protein structures\textsuperscript{64} as:

$$D_i = \frac{1}{N} \sum_{j=1}^{N} \frac{E_i}{|d_j|^2}$$

Here, $E_i$ is the energy contribution of atom $i$, $d_j$ is the displacement vector of atom $j$, and $N$ is the number of atoms in the molecule.

The alterations of signal reception and transmission properties in Kindlin mutants were computed using Markov’s chain model in DynOmics\textsuperscript{65}. We used Phos3D\textsuperscript{66} to examine the
effects on phosphorylation of experimental validated kindlin phosphorylation sites using 3D pdb coordinates.

Hydrophobic (Isoleucine, Leucine, Valine) clusters predicted to confer stability to proteins were visualized based on the Contacts of Structural Units (CSU) algorithm\(^{57}\), creating a Fibonacci-styled residue matrix and a subsequent graph plotting hydrophobic clusters for all mutant structures. Electrostatic interactions, which conferring stability and structural compactness to proteins, were computed by predicting the extent of charge segregation (\( \kappa \)) and the fraction of charged residues in a sequence (FCR) for all mutant variants\(^{57}\).

**Gene Ontology and Pathway Enrichment Analysis**

To locate associated cellular compartments, pinpoint related molecular functions, and outline linked cellular processes, we performed gene ontology using BioGRID\(^{4,57}\) to profile the high-throughput physical interactome of FERMT1, FERMT2, and FERMT3 respectively. The interactome components were restricted to proteins found in *Homo sapiens* only. Using STRING (v. 11.5)\(^{68}\), the full STRING network type with a medium confidence, minimum interaction score of 0.400 was employed to indicate both functional and direct physical protein associations.

Ensembl ID sets for FERMT1, FERMT2, and FERMT3 were used to predict and perform pathway enrichment analysis. Gene ontology (GO) molecular functions were computed as a function of fold enrichment. The Kindlin-associated mechanoochemical proteins were identified by a meta-analysis from text-mined articles. Hallmark genes were identified from MsigDB (http://www.gsea-msigdb.org/gsea/msigdb/index.jsp). The co-alteration analysis was performed for these mechanosensitive proteins and hallmark genes and the extent of co-alterations were represented using the term mean co-alteration dynamics which we have defined as:

\[
X = \sum_{N} A\% \cdot \sum_{N} U\%
\]

Here, \( X \) = mean co-alteration dynamics of a gene set; \( A\% \) = percentage of samples altered; \( U\% \) = percentage of samples unaltered; \( N \) = number of genes in set.

Pathway alterations were applying gain-of-function and loss-of-function of pathway associated genes when Kindlin is altered for checking activation and inhibition respectively. Global percentage of pathway activity for a particular pathway and for a particular Kindlin was calculated as:

\[
Global \ percentage = \left( \frac{No. of \ cancer \ activation/inhibition}{No. of \ types \ of \ cancer} \right) \times 100 \%
\]

The detailed methodology EMT-specific signalling modules and biological pathways were analysed using EMTome\(^{69}\).

**Partner-Specific Interaction Analysis**

Direct physical protein associations filtered from STRING (v. 11.5)\(^{68}\) were docked with their respective partner Kindlin employing a hybrid algorithm of template-based and *ab initio* free modelling and docking\(^{28}\). The computed docking scores, and subsequent curation of fold change in docking energy relative to the wild type enabled the prediction of the ligands’ and
the receptor’s binding affinity for all cancer mutation variants. The effects of cancer-associated substitution mutations on the monomeric Kindlin’s ability to form a dimer structure were also predicted via symmetric C2 docking\textsuperscript{70} of the monomer using the same algorithm. The binding affinity of dimerization was calculated as:

$$\Delta BA(\text{dimerization}) = BA(\text{Mutant}) - BA(\text{WT})$$

BA = binding affinity, WT= wild type. Binding affinities are represented in Kcal/mol unit. If $\Delta BA_{\text{dimerization}} = (+) \text{ ve}$, it suggests a destabilization and unfavourable while $(-) \text{ ve} \Delta BA_{\text{dimerization}}$ suggests a stabilization and is favourable.

The fold change in interactor specific interaction affinity was calculated as:

$$\text{Interaction Fold Change} = (\frac{\text{IE(mutant)}}{\text{IE(WT)}})$$

WT=wild type; IE=mutant; Fold change of 0.1 = 24 KJ/mol for FERMT1, 22.7 KJ/mol for FERMT2, 23.1 KJ/mol for FERMT3. Interaction fold change $>1$, increased interaction; Interaction fold change $<1$, decreased interaction; Interaction fold change $=1$, affinity same as the wild-type.

Further details of datasets used, copy number variation analysis, methylation analysis, classification of stabilizing and destabilizing mutants, meta-analysis of Kindlin-associated mechanochemical signalings, calculation of signal transduction properties and cancer-specific pathway analysis are given in Supplementary methodology.

Statistical Analysis
Statistical analyses were performed using R version-4.2.1, R version-4.0.3 (http://cran.r-project.org/) and OriginPro (https://www.originlab.com/). Routine statistical tests were employed to validate statistical significance. This includes the Log rank p-test and Cox proportional-hazards model for KM plots with 95% confidence intervals, Kruskal-Wallis for comparing the survival corresponding to mutations in different Kindlins. While comparing two groups, we used unpaired two-tailed t-tests. One-Way ANOVA and Bonferroni post-hoc test was employed to calculate statistical significance within cancer subtypes of the same cancer type (cut-off $\leq 0.05$). Significance cut-off for p-value was taken $<0.05$. Z-scores to study gene expression alterations were considered for samples with a cut-off of $\pm 1.96$ corresponding to p-value $<0.05$. For significant data of fold enrichment in $-\log_{10}(\text{FDR})$ scale, cut-off for FDR was taken at less than 0.05. Spearman’s Coefficient correlation was used to validate correlation between two non-parametric variables, where +1 indicates highest correlation, -1 indicates anti-correlation, and 0 indicates no correlation. The nature of data distribution was verified and represented in terms of the Shapiro-Wilk normality test and skewness. Bars, wherever used, indicate standard error of mean.

Data availability
Mutation data is available from COSMIC (https://cancer.sanger.ac.uk/cosmic). Normal tissue gene expression data is available at GTEx (https://gtexportal.org/home/). miRNA data is available at miRDB (http://www.mirdb.org/) and their cancer-specific alteration data (TCGA set) is available at miRCancer (http://mircancer.ecu.edu/). Mutation-specific structural data, phosphorylation data and Mechano-chemical protein meta-analysis generated data are available in the supplementary information file. Other data generated during this study are available from the corresponding authors upon reasonable request.

**Code availability**

Codes generated during this study are available from the corresponding authors upon reasonable request.

**Author Contributions**

D.C and S.H designed the project. D.C handled the COSMIC dataset. A.M handled the TCGA dataset. R.B and S.W handled the PCAWG dataset. D.C, A.M, R.B, and S.W analysed the data. D.C, A.M, R.B, and S.W performed the experiments. D.C, A.M, R.B, and S.W analysed the experimental data. D.C, R.B and A.M built the codes. D.C, A.M, R.B, and S.W performed the meta-analyses. D.C, A.M, R.B, and S.C drafted the manuscript with input from all the authors. D.C, A.M, R.B and S.W participated in figures preparation. S.H and S.C reviewed the manuscript. All authors have read and approved the final version of the manuscript.

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**Conflict of Interest**

The authors declare no conflict of interest.

**References**


Fig 1. Pan-cancer framework of various alterations and expression change of Kindlin family proteins along with their clinical significance. a. Prevalence of kindlin proteins alterations in
different types of cancers. The color codes indicate types of alterations as shown in the figure. 
b. Expression levels of kindlin mRNA in different types of cancer samples with respect to the 
corresponding normal tissues in terms of Z-score (±1.96 cut-off, p-value < 0.05). ***, p-value <0.0005; **, p-value <0.005; *, p-value <0.05; absence of * = no significance or absence of 
sample in a cohort, paired p-values were obtained by student t-test. c. Expression levels of 
kindlin proteins in different types of cancer samples with respect to the corresponding adjacent 
normal tissues in terms of Z-score (±1.96 cut-off, p-value < 0.05). ***, p-value <0.0005; **, p-value <0.005; *, p-value <0.05; absence of * = no significance or absence of sample in a 
cohort, paired p-values were obtained by student t-test. d. Changes in mRNA expression level 
with sample specific mutation count. The solid lines represent the regression line each case. 
Spearman’s correlation: FERMT1, ~-0.25 (P-value= 4.184 x 10^{-15}); FERMT2, ~ -0.24 (P-
value= 1.356 x 10^{-14}); FERMT3, ~ 0.16 (P-value= 6.867 x 10^{-7}). (e-g). Comparative quartile 
overall survival probability curve as Kaplan-Meier plot of high and low mRNA expression 
sample group in case of e. FERMT1 (Logrank P value, 0; Hazard ratio, 1.9); f. FERMT2 
(Logrank P value, 0.81; Hazard ratio, 1); g. FERMT3 (Logrank P value, 3.336 x 10^{6}; Hazard 
ratio, 1.3). (h-j). Comparative quartile disease-free survival probability curve as Kaplan-
Mayer plot of high and low mRNA expression sample group in case of h. FERMT1 (Logrank 
P value, 0.00011; Hazard ratio, 1.2); i. FERMT2 (Logrank P value, 3.0 x 10^{-10}; Hazard ratio, 
1.4); j. FERMT3 (Logrank P value, 0.031; Hazard ratio, 1.1). Quartile cut-off high: 75%, low: 
25%; dotted lines corresponding to the survival probability lines represent 95% CI. 100-month 
cut-off survival time is designated as dotted line (parallel to y-axis) is shown in each image.
Fig 2. Characterization of Kindlin mutations in patient cohort. a. Mutation frequency (in % samples) of kindlin coding mutations across various types of tumor samples (Colors are representative of types of cancer in lower horizontal bar). Different mutation types are also
represented with different colors in respective patient samples. b. Residue specific distribution of somatic mutations across all three kindlins. Types of mutations and their respective color coding are presented with respective images. Below the corresponding sequence-specific mutation frequency graphs, kindlin domain organization is given. Each color in these represents specific domains. c. Comparative plot of Z-score values for differentially expressed mutated (non-coding) kindlin transcripts (significance cut-off z-score ±1.96, p-value < 0.05). Different color-coded bars indicate different kindlins. Cancer-specific impact of the mutations are denoted by contrasting border colors of the bars. Positions of the non-coding mutations are indicated in text corresponding to each bar. d. Comparative Survival time versus survival probability curve for kindlin mutated (red) and non-mutated (blue) sample cohort. (p value = 0.0003, Hazard ratio, 1.932). e. Comparative Survival time versus survival probability curve for different kindlin mutated sample cohort. FERMT1, pink; FERMT2, yellow; FERMT3, purple; (Kruskal-Wallis rank sum p value: FERMT1-FERMT2, 0.0058; FERMT2-FERMT3, 0.0002; FERMT1-FERMT3, 0.2945) f. Tumor stage specific mutations in kindlins. The area of each quadrilateral and corresponding size of tumor stage name text indicates the amount of alteration. g. Metastasis stage specific mutations in kindlins. The area of each quadrilateral and corresponding size of metastatic stage name text indicates the amount of kindlin alterations involved.
Fig 3. Stability analysis of mutated Kindlins. Only missense mutations (either single or multiple mutation variants) are considered here. (a–c). $\Delta \Delta G$ vs $\Delta \Delta S$ plots to determine stability of the mutants against their flexibility for a. kindlin1 (FERMT1) b. kindlin2 (FERMT2) and c.
kindlin3 (FERMT3). ΔΔG values and ΔΔS values are presented in KJ/mol and KJ/(mol.K) unit respectively. The plots are divided into four quadrants (Q1-Q4) according to the nature of four different mutant population. Q1, increased stability and flexibility (red); Q2, increased stability and decreased flexibility (green); Q3, decreased stability and flexibility (blue); Q4, decreased stability and increased flexibility (purple). Regression lines are shown in gray. d. Mapping kindlin mutations according to their plausible impact on functionalities. Values <0.05 indicates loss-of-function mutants, rest are neutral mutants. (e-f) Stability changes in mutated protein dimer structures with respect to wildtype in Sum ΔΔG values (KJ/mol). d. kindlin1 (FERMT1) e. kindlin2 (FERMT2) and f. kindlin3 (FERMT3).
Fig 4. Functional impact of frameshift mutations of Kindlins  

a. Common domain organization of kindlins as a schematic diagram.  
b. Comparison of domain organization of wildtype kindlin2 (FERMT2) with its cancer-specific indel variants (Inset: Superimposed structure of WT and K154del mutant containing all the domains show an altered orientation of various domains).  
c. Comparison of domain organization of wildtype kindlin1 (FERMT1) with its cancer-specific indel variants.  
d. Comparison of domain organization of wildtype kindlin3 (FERMT3) with its cancer-specific indel variants (Inset: Superimposed structure of WT and A643del mutant containing all the domains show an altered orientation of various domains). Differently colored domains indicate their similarity with pfam annotated domains from different organisms.
Fig 5. Mutation induced root mean squared fluctuation perturbations in Kindlins. a. Force induced normal mode trajectory of the wildtype kindlin dimers (superposed). Mobility of atoms are defined by a given scale with contrasting colors. I1, integrin binding domain 1; I2, integrin binding domain 2; P1, F0 domain of Chain A; P2, F0 domain of Chain B; The distance between I1 and I2 are given by X (Low force conformation) and X’ (High force conformation). Similarly, distance between I1(Low force)-P1 (Low force), Z; I2(Low force)-P2(Low force), Y; I1(High force)-P1 (High force), Z’; I2(High force)-P2(High force), Y’. b. Residue-wise B-factor analysis of kindlins and their three most-perturbed mutants each. Extent of redness indicates increase in B-factor. Same colored boxes are used to show similar position with altered B-factor with respect to the wildtype. Different colored boxes are used if alteration is different in some mutant(s) or in different positions. Numbers represent the start and end amino acid residue index in each chain.
Fig 6. Mutation induced perturbation in binding affinity of Kindlins. Change in dimerization affinity with respect to the wildtype is calculated as $\Delta \Delta BA_{dimerization}$ (KJ/mol) and plotted for all the significantly perturbing mutants of FERMT1 (a), FERMT2 (b) and, FERMT3 (c). (d-f) represents mutation induced fold change (mutant/WT) in binding affinity of the mutant-direct binder interactions of FERMT1 (d), FERMT2 (e), and FERMT3 (f). Fold change of 0.1 = 24 KJ/mol for FERMT1, 22.7 KJ/mol for FERMT2, 23.1 KJ/mol for FERMT3. Interaction fold change>1, increased interaction; Interaction fold change<1, decreased interaction; Interaction fold change=1, affinity same as the wild-type. (Reference for cellular significance: energy stored within one high-energy molecule (ATP)~30 KJ/mol.)
Fig 7. Co-alteration analysis of direct interactors of Kindlins, cancer hallmark genes and Kindlins associated mechanoochemical signal proteins and their effect on major cancer-associated pathways. (a-c). The alteration frequencies (in percentage) of direct interactors of
corresponding kindlins in respective kindlin-altered and non-altered cancer samples (PCAWG/ICGC/TCGA cohort): FERMT1 (a), FERMT2 (b), and FERMT3 (c). d. The alteration frequencies (in percent) of major cancer hallmark genes with respective kindlin altered and unaltered cancer samples (PCAWG/ICGC/TCGA cohort). Different colored chords connecting hallmark genes and status columns indicate respective hallmark genes. The breadth of the chords is directly proportional to the number of altered or unaltered samples. e. The alteration frequencies (in-percent) of major kindlin associated proteins involved in mechanochemical signalings with respective kindlin altered and unaltered cancer samples (PCAWG/ICGC/TCGA cohort). The size of the circles is indicative of percentage altered/unaltered samples. The colors represent p-values as given aside the image. Significance cut-off p-value<0.05. f. Influence of kindlin (FERMT1, 2, 3) alterations in signature cancer-related pathways in 32 different cancer types. CCP, cell cycle progression; DDR, DNA damage response; EMT, Epithelial-mesenchymal-transition; AR, Androgen receptor; ER, Estrogen receptor; RTK, Receptor tyrosine kinase pathway. A pie represents 100%.
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