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Pan-Cancer Analysis of ARHGAP35 Reveals Prognostic Relevance in Breast and Cervical Cancers.

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ABSTRACT

Rho GTPase-activating protein ARHGAP35 has been linked to tumour progression, but its pan-cancer activity is not well understood. The current study has explored gene expression, mutation patterns and clinical relevance of ARHGAP35 in several cancers, using TCGA datasets. Analysis of expression showed that TGCT, KIRP and UCEC were significantly down regulated and BRCA, ESCA, KICH and LAML were highly expressed. Oncoprint findings revealed that genomic changes on ARHGAP35 are not common, with less than 2 percent of cases reported, and that amplification and truncating mutations are the most common. Mutation distribution analysis was used to show that there was a dispersed pattern with no hotspots but moderate clustering was found in the middle of the protein. Functional domain mapping implied that a relatively smaller number of mutations in conserved areas would occur, but variants in the RhoGAP domain can have an effect on tumour-associated signalling. Genetic modification of ARHGAP35 showed survival analysis results in worse prognosis of breast and cervical cancers, with decreased median survival rates of altered groups. Comprehensively, the results indicate that ARHGAP35 can act more as a tumour suppressor, and that loss-of-function mutations may occur in some cases, which lead to cancer development. Overall, these results indicate the potential of ARHGAP35 as a prognostic biomarker and offer preliminary clues to its potential role in cancer biology.

Keywords: Tumor progression, ARHGAP35, Rho GTPase signalling, Tumor suppressor

1. INTRODUCTION

Cancer is an umbrella term for a multitude of genetic diseases caused by the aggregation of harmful DNA mutations and epigenetic alterations that allow uncontrolled cancer cell proliferation (Gopal et al., 2018; Krinio Giannikou et al., 2016). Currently, cancer is the second most common cause of death worldwide. It is characterized by 6 main hallmarks: sustaining proliferative signaling, evading growth suppressors, activating invasion and metastasis, enabling replicative immortality, inducing angiogenesis, and resisting cell death (Wang et al., 2020). Current treatments for cancer include chemotherapy, hormone therapy, hyperthermia, immunotherapy, photodynamic therapy, radiation therapy, blood stem cell transplant, surgery, and targeted therapy, though all are not completely effective (Reardon et al., 2018; Eaton et al., 2021). Some limitations of current treatments include drug resistance, high toxicity to healthy cells, and

tumor heterogeneity (Lecuyer et al., 2025). Cancer genomics aims to study and understand the genetics behind tumor cell proliferation, the difference in gene expression between a host cell and tumor cell, and the evolution of the cancer genome (Gopal et al., 2018). Currently, there are two known types of genes responsible for cancer progression: oncogenes and tumor suppressor genes. Oncogenes are mutated versions of proto-oncogenes that initiate improper and unregulated cell proliferation (Wang et al., 2020). Proto-oncogenes generally regulate the cell cycle in order to prevent perpetual cell proliferation and division (Parasuraman et al., 2017). Tumor suppressor genes, on the other hand, prevent unregulated cell growth by blocking pathways that lead to this proliferation in the first place. When mutated, they fail to block the cancerous pathways, which also causes unregulated cell proliferation (Wang et al., 2020). Thus, it is crucial to identify prognostic biomarkers in order to target them early and prevent them from creating cancerous mutations (Zhao et al., 2014). A prognostic biomarker is used to define the probability of a clinical complication, such as disease recurrence or disease progression.

This research paper aims to discuss another gene that promotes tumor progression, specifically the ARHGAP35 gene (Héraud et al., 2019). ARHGAP35, also known as GAP1 or GRLF1, encodes a Rho GTPase-activating protein. It serves as a critical regulator of multiple fundamental cellular processes, including cell cytoskeleton organization, cell migration, adhesion, cytokinesis, and neural development (Héraud et al., 2019; Rodríguez-Fdez & Bustelo, 2021). As a RhoGAP family member, ARHGAP35 primarily functions by inactivating RhoA, a small GTPase protein that plays a central role in regulating actin cytoskeletal dynamics and downstream signaling cascades involved in cell morphology and motility (Bidaud-Meynard et al., 2017; Ponik et al., 2013). Dysregulation of this RhoA-mediated pathway has been implicated in aberrant cellular behavior consistent with malignant transformation (Sun et al., 2022; Kang et al., 2025). Although ARHGAP35 has already been found to play a contributory role in uncontrolled tumor growth in a number of different cancer types, the underlying molecular pathways through which its mutation or changed expression leads to oncogenesis are not well described (Chen et al., 2019; Li et al., 2021) The few studies carried out so far have provided partial and even conflicting results and there are still large gaps in our knowledge of how ARHGAP35 dysfunction leads to tumor formation, progression and metastasis (Sun et al., 2022; Zhao et al., 2014). With the gene playing a central role in cytoskeletal remodelling and cell migration, which are processes that are fundamentally related to cancer invasiveness, a closer examination of the role of ARHGAP35 in tumorigenesis is not only justified but required (Kang et al., 2025; Monaghan-Benson et al., 2018). The aim of this paper is to fill these gaps by summarizing existing evidence, evaluating the trends of ARHGAP35 mutation in diverse cancer types, and assessing its capacity to become a new prognostic biomarker and treatment target.

2. MATERIALS & METHODS

2.1 Data Access:

The Cancer Genome Atlas (TCGA) data was accessed through two complementary platforms, cBioPortal for Cancer Genomics (https://www.cbioportal.org/) and Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia2.cancer-pku.cn/#index). These platforms were used to analyse gene expression, Oncoprint, genetic alteration, and survival analysis of ARHGAP35 across different cancer types.

2.2 Data selection:

Cancer genomics datasets that were publicly available were downloaded from the cBioPortal for Cancer Genomics platform (https://www.cbioportal.org/) to examine the contribution of ARHGAP35 in various cancer types. The following criteria were used for the selection of datasets: (i) comprehensive genomic alteration data, such as mutations and copy number alterations, were available, (ii) clinical and survival data were available, (iii) the datasets were large-scale pan-cancer studies, and (iv) the datasets were publicly accessible with standardized annotation and quality control. The studies excluded from the analysis did not have full genomic or clinical data.

For the pancancer analysis, a total of 12 pan-cancer analyses (148,790 patients and 156,010 samples) were included. The datasets chosen included: Cancer Therapy and Clonal Hematopoiesis (MSK, Nat Genet 2020), China Pan-cancer (OrigiMed, Nature 2022), Metastasis Solid Cancers (UMich, Nature 2017), MSK MetTropism (MSK, Cell 2021), MSK-CHORD (MSK, Nature 2024), MSK-IMPACT 50K Clinical Sequencing Cohort (MSK, Cancer Cell 2026), MSK-IMPACT Clinical Sequencing Cohort (MSK, Nat Med 2017), MSS Mixed Solid Tumors (Broad/Dana-Farber, Nat Genet 2018), Pan-cancer Analysis of Whole Genomes (ICGC/TCGA, Nature 2020), SUMMIT-Neratinib Basket Study (Multi-Institute, Nature 2018), TMB and Immunotherapy (MSK, Nat Genet 2019), Tumors with TRK Fusions (MSK, Clin Cancer Res 2020). These data sets were chosen as being representative of a variety of different types of cancers and for their high level of genomic profiling data for pan-cancer analysis of ARHGAP35.

Thirty-six breast cancer studies consisting of 17,758 samples from 16,005 patients and four cervical cancer studies with 1,093 samples from 1,089 patients were chosen based on the same inclusion criteria as above.

2.3 Gene Expression Analysis

The Gene Expression Profiling Interactive Analysis 2 (GEPIA2) web server was used for gene expression analysis for ARHGAP35 that uses the RNA sequencing data from The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) projects. The gene symbol “ARHGAP35” was subjected to GEPIA2 search and analyzed its expression levels between tumor and normal tissues.

The default GEPIA2 normalization pipeline (log2(TPM +1) transformed RNA-seq data) was used for the expression comparison between tumor and normal tissues. Differences between tumor and normal tissues were considered statistically significant with default cutoff values of |log2 fold change| ≥ 1 and p-value<0.01.

2.4 Oncoprint Analysis

The cBioPortal for Cancer Genomics was used to analyze and visualize ARHGAP35 genomic alteration data across all cancers. The gene symbol “ARHGAP35” was typed in under the “Query by Gene” section to fetch the alteration information from all the selected tumor studies.

Genomic alteration profile of ARHGAP35 was plotted in an OncoPrint to visualize the profile in tumor samples. In the OncoPrint, each column was one patient sample; each row was one gene (in this case, ARHGAP35). Different types of genetic alterations (missense mutations, truncating mutations, gene amplifications, deep deletions and multiple alterations) were represented by different color codes

automatically generated by the platform. Genomic alterations were detected in at least one gene in the sample, which was then classified as 'altered'; otherwise, it was classified as 'unaltered'.

2.5 Analysis of Distribution of Somatic Mutations

Somatic mutation distribution was examined across the protein sequence in ARHGAP35 in cBioPortal. The ARHGAP35 somatic mutation data was obtained from all tumor data sets selected by querying ARHGAP35. The built-in module for visualising mutations was used to create a lollipop plot to assess the distribution and number of mutations across the protein sequence. The platform automatically annotated the mutation types using the standard genomic classification criteria; they were missense, truncating, splice-site and in-frame mutations.

2.6 Analysis of Survival Prognosis

The "Comparison/Survival" module of cBioPortal was used for survival analysis. Genomic alterations such as mutations and copy number changes were examined in the genomes of the patients and divided into an "altered" and "unaltered" category. Altered groups are those patients who have genetic change in ARHGAP35 and unaltered groups are the patients with no genetic changes in ARHGAP35 of tumor samples. Overall survival was compared between the two groups by Kaplan–Meier survival curves. The log rank test was performed in the cBioPortal platform to determine the statistical significance. A p-value <0.05 was considered statistically significant.

2.7 Genetic Alteration Analysis

The "Cancer Types Summary" module of cBioPortal was used for genetic alteration analysis of ARHGAP35. The incidence and patterns of mutations, amplifications and deep deletions were studied in various cancer types. Alteration frequencies were automatically calculated based on the percentage of samples with at least one detectable alteration of the gene. Copy number alteration (CNA) data were analyzed by categorization of CNA as provided in cBioPortal. The platform's statistical summaries and visualization outputs were employed to interpret alteration patterns within tumor cohorts.

3 RESULTS

3.1 Gene expression analysis:

The results of the expression analysis showed a heterogeneous, but biologically meaningful pattern of ARHGAP35 dysregulation by cancer type. A significant reduction in ARHGAP35 expression was detected in tumour tissues compared with normal tissues in Testicular Germ Cell Tumors (TGCT), Kidney Renal Papillary Cell Carcinoma (KIRP) and Uterine Corpus Endometrial Carcinoma (UCEC) indicating a possible loss of the regulatory function during tumour progression in these malignancies. Because ARHGAP35 is known to regulate Rho GTPase signaling, cytoskeletal organization and cell migration, decreased expression potentially might lead to increased cell proliferation and invasive capabilities by disrupting normal control of cellular signaling. This downregulation behaviour is similar to

what is typically found in tumour suppressor genes, in which reduced expression can de-inhibit oncogenic pathways.

In contrast, high expression of ARHGAP35 is seen in Breast Invasive Carcinoma (BRCA), Esophageal Carcinoma (ESCA), Kidney Chromophobe (KICH), and Acute Myeloid Leukemia (LAML). The differential expression pattern implies that the function of ARHGAP35 might vary according to the context and be affected by tissue-specific molecular context. High expression in some cancers could be a compensatory mechanism of the cells to oncogenic stress or changes in signaling pathways, but not a direct oncogenic role. Interestingly, even though ARHGAP35 was overexpressed in BRCA, further survival analyses showed associations with prognostic outcomes, reflecting the fact that expression alteration does not necessarily provide a full description of the biological functions of ARHGAP35. Together, these results show an expression pattern in cancer, and at the same time have features characteristic of a mostly tumour-suppressive role in various cancers (Figure 1).

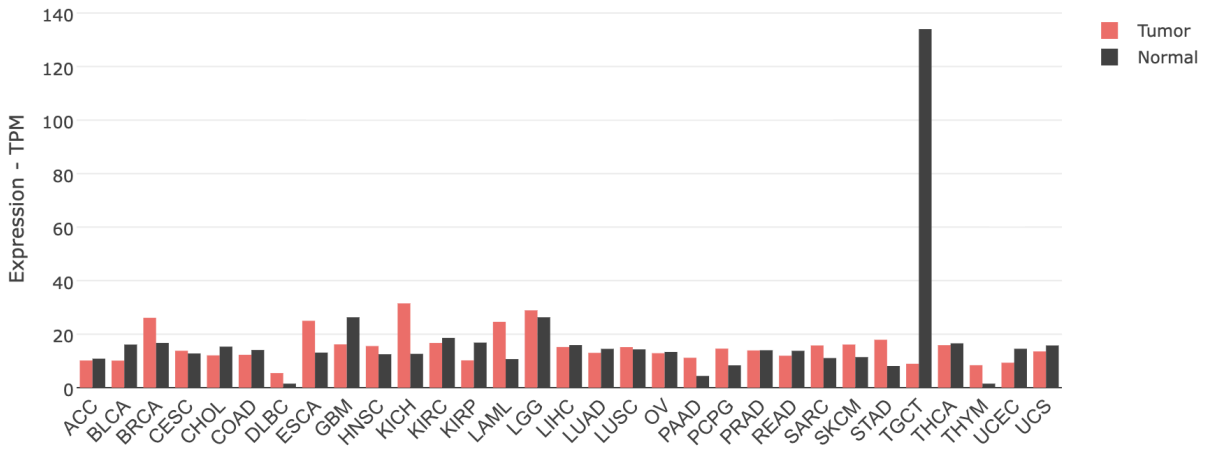

Fig. 1. Gene expression analysis. The bar graph indicates ARHGAP35 expression (transcripts per million) in tumor tissues and their corresponding control tissue.

3.2 Oncoprint analysis:

Genomic alterations in ARHGAP35 were assessed across multiple pan-cancer datasets using OncoPrint visualization (Figure 2). Overall, alterations in ARHGAP35 were rare, occurring in less than 2% of the analyzed samples. The majority of genetic alterations consisted of amplification, truncation mutation (putative driver) and only a few missense variants of unknown significance.

Fig. 2. Oncoprint analysis. Genomic alterations in ARHGAP35 across multiple pan-cancer datasets.

3.3 Genetic alteration analysis:

The genetic alteration status of ARHGAP35 in different tumor samples of the TCGA cohorts were analyzed. Figure 3 indicates that the highest alteration frequency of ARHGAP35 appears for uterine endometrial carcinoma, endometrial cancer, lung cancer, and skin cancer patients with "mutation" as the primary type. The "amplification" type of CNA is the secondary type in lung cancer and cervical cancer cases.

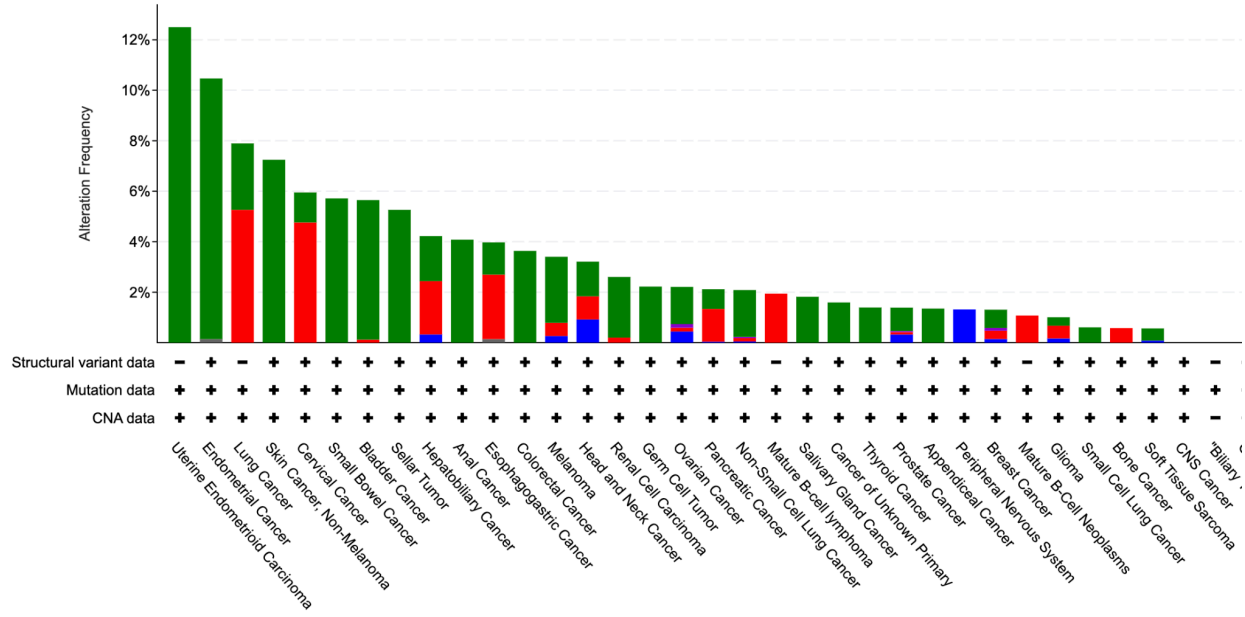

Fig.3 Genetic alteration analysis: The bar graph indicates alteration frequency of ARHGAP35 in different cancer types.

3.4 Analysis of distribution of somatic mutations in ARHGAP35

The lollipop plot analysis showed that somatic mutations in ARHGAP35 are distributed across the entire protein sequence and are not clustered around any hotspot mutation as are those of classical oncogenes. Rather, the mutations were randomly distributed at low frequencies and had multiple truncating or frameshift mutations, especially in the middle part of the molecule (~900 – 1000 aa), including those that had been seen before, such as K945Rfs and N946Efs. This scattered mutational structure is more representative of the genomic behaviour of tumour suppressor genes, which are often inactivated by a range of different loss-of-function mutations, rather than by activating hotspot substitutions.

The relatively low number of mutations found in the N-terminal Ras-association (RA) domain and FF domains could suggest that this is a structurally or functionally constrained area where mutations are not well tolerated. By contrast, the C-terminal RhoGAP domain showed a moderate number of variants, indicating its possible functional relevance in signaling dysfunction in cancer. As the RhoGAP domain has a central role in controlling Rho family GTPase activity, mutations in this domain could affect cytoskeletal regulation, cell adhesion, and migratory control thus promoting tumor progression.

It is important to note that several mutations occurred within regions that overlap with or are adjacent to sites predicted for phosphorylation or other post-translational modification (PTM) with emphasis in the C-terminal region. Such changes could disrupt regulatory signaling pathways, protein stability, and/or protein–protein interactions, and could result in changes to downstream pathways. The lack of dominant hotspots for activating mutations, combined with the abundance of truncating and regulatory region mutations, strongly indicates that ARHGAP35 could be a context-dependent tumour suppressor gene whose inactivation plays a role in tumorigenesis by either partial or complete loss of normal regulatory function (Figure 4).

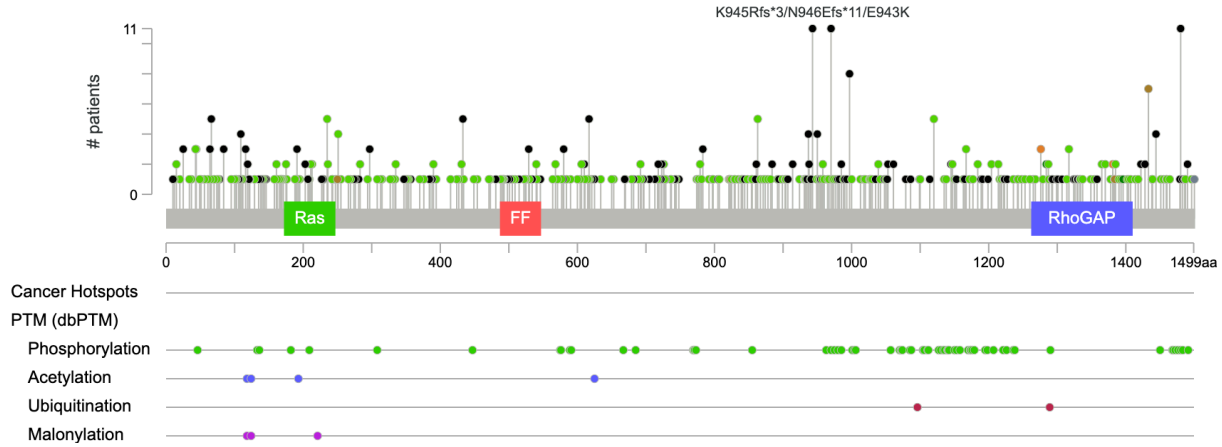

Fig.4 distribution of somatic mutations in ARHGAP35**:** The lollipop plot illustrates the distribution of somatic mutations in ARHGAP35 along the protein sequence across cancer samples.

3.5 Role of ARHGAP35 in breast cancer:

The genetic alteration status of ARHGAP35 in different breast tumor samples of the TCGA cohorts were analyzed. Figure 5 indicates that the highest alteration frequency of ARHGAP35 appears for breast invasive cancer patients with “mutation” as the primary type. The “amplification” type of CNA is the secondary type in breast invasive cancer. Figure 6 shows the probability of overall survival in an altered and unaltered group using cBioPortal platform. Kaplan–Meier survival analysis was used to assess the prognostic value of ARHGAP35 changes in the cancer population by comparing the overall survival between ARHGAP35 altered and non-altered groups. The number of patients included in the analysis were 103 patients in the altered group and 6,878 patients in the unaltered group. The survival curves showed that patients with ARHGAP35 alterations had shorter median OS than those without ARHGAP35 alterations. The median overall survival was 100.62 months (95% CI: 50.70–NA) for the altered group, and 148.80 months (95% CI: 142.43–158.63) for the unaltered group. This found about 48 months decrease in median survival period indicates that ARHGAP35 changes may have a negative effect on prognosis. The hazard ratio (HR) of the unaltered group compared to the altered group was 0.693 (95% CI: 0.414–1.160). HR < 1.0 means that the unaltered group was at a lower risk of death than the altered group. Specifically, patients with no alterations of ARHGAP35 had an estimated 30.7% decrease in the risk of death compared with patients with alterations in ARHGAP35. In the altered group, the reciprocal HR was 1.442 (95% CI: 0.862–2.413), suggesting an increasing risk of mortality for patients with ARHGAP35 alterations. The log-rank test, however, showed a p value of 0.0928, which was not considered statistically significant ($p < 0.05$). Moreover, the 95% confidence intervals for the hazard ratio also straddled 1.0, suggesting that the difference in survival observed was somewhat uncertain. Thus, while the survival trend indicates poorer prognosis in patients with ARHGAP35 alterations, as yet statistical evidence to confirm ARHGAP35 as an independent prognostic biomarker in this patient population is lacking. This could be because the altered group (n = 103) was relatively small compared to the unaltered group (n = 6,878), which may limit the statistical power and widen the confidence interval. However, the trend of decreased survival in the modified group is biologically significant and could be further substantiated by additional independent study cohorts and multivariate survival analysis.

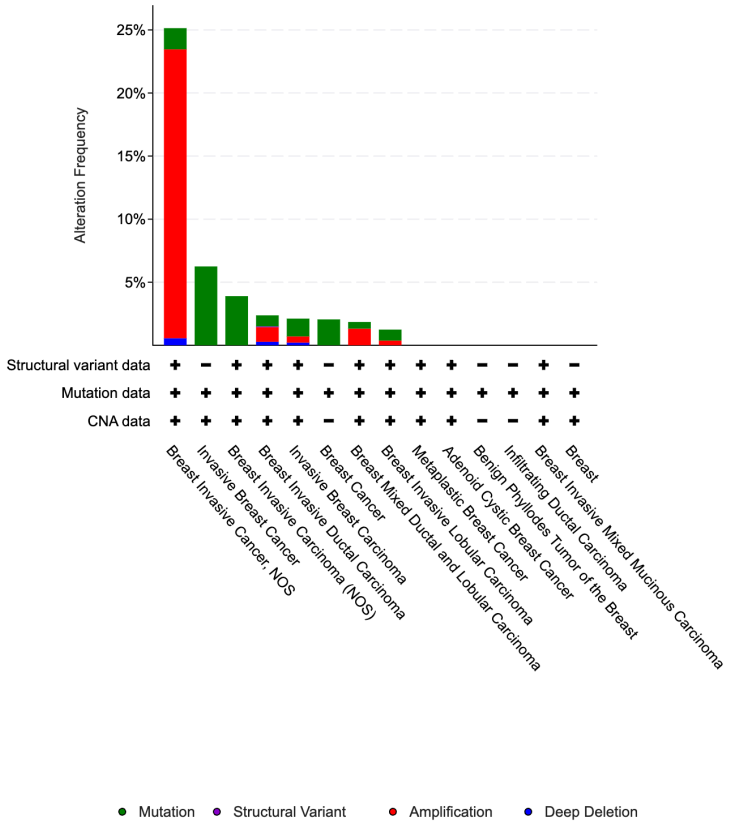

Fig.5 Genetic alteration analysis: The bar graph indicates alteration frequency of ARHGAP35 in different brain/CNS related tumor types.

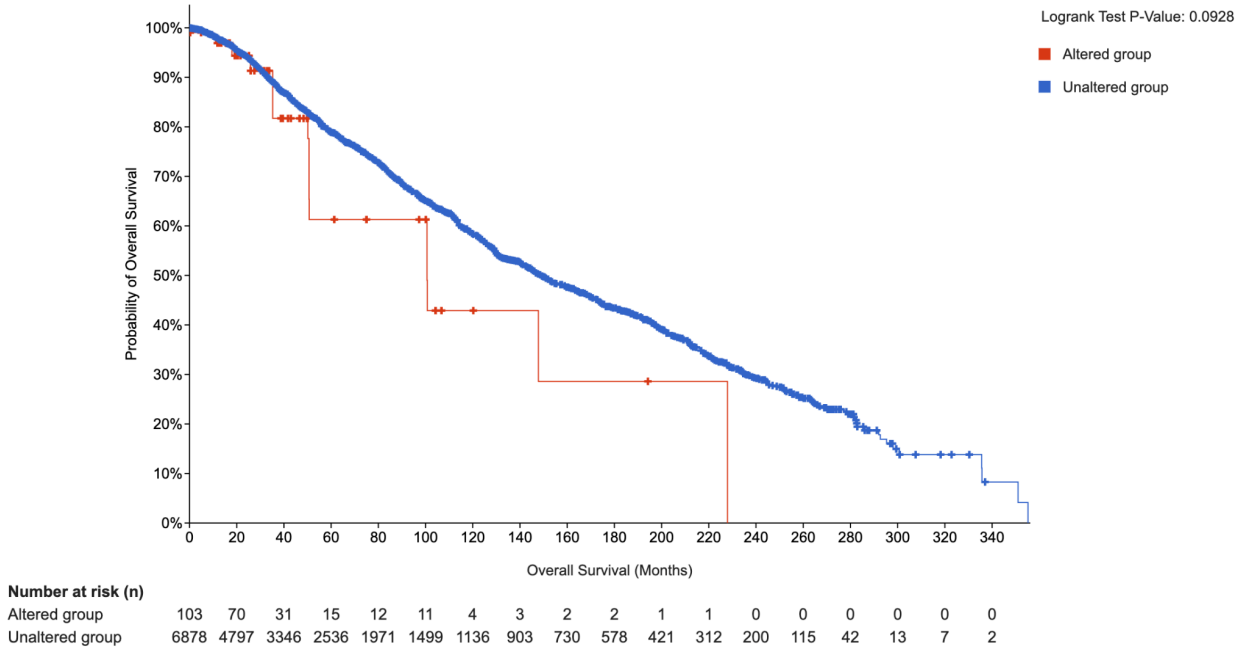

Fig. 6. Analysis of survival prognosis. The Kaplan-Meier plot indicates probability of overall survival in unaltered and altered groups.

3.6 Role of ARHGAP35 in cervical cancers:

The genetic alteration status of ARHGAP35 in different cervical tumor samples of the TCGA cohorts were analyzed. Figure 7 indicates that the highest alteration frequency of ARHGAP35 appears for cervical squamous cell carcinoma and cervical cancer patients with “mutation” as the primary type and the “amplification” type of CNA is the secondary type in both. Figure 8 shows the probability of overall survival in an altered and unaltered group using cBioPortal platform. Patients with ARHGAP35 alterations had significantly worse OS than those without the alterations (p = 0.002, Kaplan–Meier survival analysis). The median overall survival was 100.62 months (95% CI: 50.70–NA) in the altered group and 148.80 months (95% CI: 142.43–158.63) in the unaltered group. The unaltered had a hazard ratio of 0.693 (95% CI: 0.414–1.160) with an estimated 30.7% decrease in the risk of mortality compared to the altered group. On the other hand, patients with ARHGAP35 abnormalities showed a tendency for a higher risk of death (HR = 1.442, 95% CI: 0.862–2.413). The survival curves indicated an unfavorable prognostic impact of ARHGAP35 alterations, but the association was not statistically significant (log-rank p = 0.0928). The lack of statistical significance could be due to the small number of altered cases and the low number of events. However, the steady downward survival rates suggest that ARHGAP35 changes might be of prognostic significance and should be explored in larger, independent patient groups.

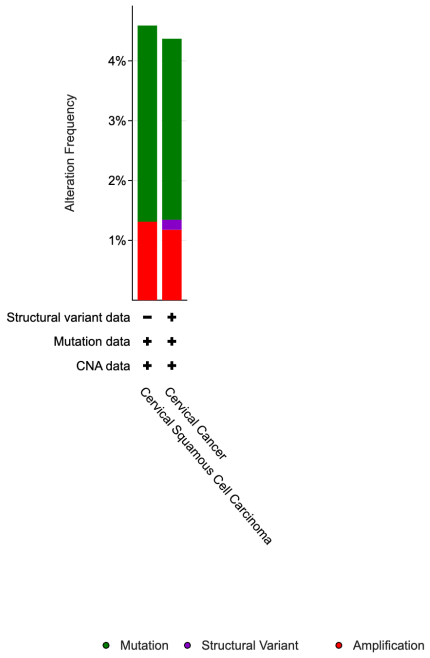

Fig.7 Genetic alteration analysis: The bar graph indicates alteration frequency of ARHGAP35 in different cervical cancer types.

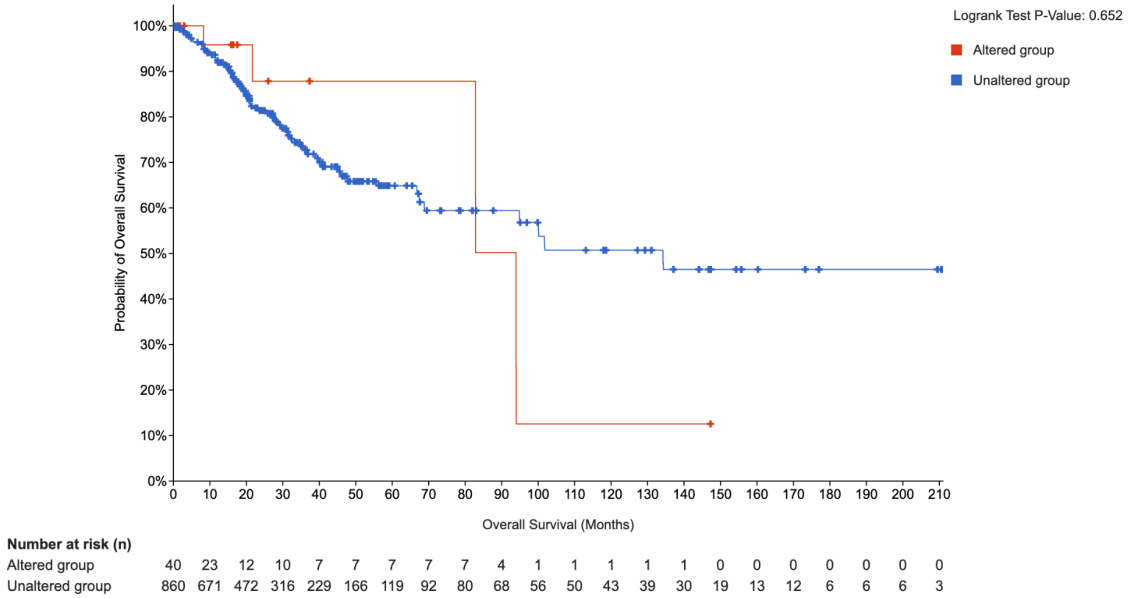

Fig. 8. Analysis of survival prognosis. The Kaplan-Meier plot indicates probability of overall survival in unaltered and altered groups.

4. DISCUSSION

ARHGAP35 (Rho GTPase Activating Protein 35) encodes a key negative regulator of Rho family GTPases, which function as molecular switches controlling cytoskeletal dynamics. ARHGAP35 is the gene that encode the p190A RhoGAP, which is a regulator of Rho-family GTPase signalling that controls cytoskeletal dynamics, contact inhibition and Hippo pathway activity all of which are involved in tumour progression (Frank et al., 2018; Ouyang et al., 2020). There is a wide range of variation in progression and therapeutic response in breast cancer, and cervical cancer is closely associated to continue high-risk HPV infection and still requires improved biomarkers to prognosticate and stratify (Sun et al., 2022; Zhao et al., 2014). Since Rho GTPase signalling has been identified to play a role in both breast and gynaecologic cancer invasion, metastasis, and treatment response, it is biologically important to assess ARHGAP35 levels in tumour types (Kang et al., 2025; Wang et al., 2020).

ARHGAP35 was dysregulated in the tumour, being lower in TGCT, KIRP and UCEC, higher in BRCA, ESCA, KICH and LAML in the present analysis. This trend is in line with the literature indicating that RHO-pathway genes may play pro-tumour or anti-tumour roles context dependent, and may not have a uniform role across all cancers (Héraud et al., 2023; Wang et al., 2020). Concurrently, the extended interpretation of ARHGAP35 continues to support a tumour-inhibitory role. It has been demonstrated that p190A restoration is capable of strengthening contact inhibition, activating LATS kinases, suppressing YAP activity and inhibiting tumour growth. These results are consistent with the current finding that

ARHGAP35 can be a potential tumour suppressor like protein rather than a classical oncogene (Frank et al., 2018; Ouyang et al., 2020).

The OncoPrint and mutation analysis are both consistent with previous reports. Mutations occurred rarely in general, the mutation pattern was scattered, and truncating events, frameshifts, amplification and few missense changes were frequent. This is similar to published data suggesting that numerous tumour-related ARHGAP35 mutations disrupt the RhoGAP activity and can be mostly loss-of-function events and not hotspots (Reis et al., 2022). The clustering of the central region and C-terminal RhoGAP domain involvement in the current study are also biologically reasonable, due to the tight interconnection of the protein localisation and GAP activity to the anti-tumour activity of the protein (Héraud et al., 2023).

In breast and cervical cancers, the alteration of ARHGAP35 in might relate to a reduced median overall survival, which argues in favor of clinical utility. This observation is consistent with the literature indicating that dys-regulation of Rho signalling causes aggressive behaviour, metastasis, and poor outcome in these cancers (Kang et al., 2025; Wang et al., 2020). Overall, the current evidence preliminarily suggests that ARHGAP35 may function as a context-specific, predominantly tumour-suppressive gene that may have potential prognostic relevance in breast and cervical cancer.

5. LIMITATIONS

While providing a pan-cancer evaluation of ARHGAP35, the present study has a number of limitations that will need to be considered in the interpretation of the results. First, the analyses were based on TCGA and other public data, and there are high levels of inter-dataset heterogeneity due to distinct sample collection, sequencing platforms, data processing pipelines, and patient demographics. This variability can also bring in possible batch effects and affect the uniformity of the gene expression and mutation analysis. Second, in several cancer types, matched normal tissue samples were available for only a small proportion of the samples, potentially impacting the reliability of differential expression analysis and limiting the robustness of analysis in some cancers. Third, the study did not validate the observed bioinformatics associations with experiment and functional studies.Third, the study conducted only retrospective observational bioinformatics analyses without experimental or functional validation and hence the findings were statistical associations and not direct evidence for biological mechanisms. Moreover, there was no longitudinal clinical data or treatment-specific information that would have allowed for the analysis of temporal shifts in ARHGAP35 expression, therapy response or disease progression over time. Additionally, confounding clinical variables may have affected the results of the survival analyses that were conducted, but were not fully controlled by the data sets available. Thus, the findings of this study should be considered as preliminary and exploratory, and hypothesis generating, and should be underpinned by further experimental, mechanistic and prospective clinical studies.

6. CONCLUSION

To gain insight into the potential biological and clinical significance of ARHGAP35, the expression profile and the genomic alteration profile of ARHGAP35 across several cancer types were systematically analyzed with the aid of publicly available bioinformatics data. The results showed that ARHGAP35 expression is dysregulated in a few types of cancer; cancers with decreased expression include TGCT, KIRP and UCEC, and cancers with increased expression include BRCA, ESCA, KICH and LAML. The differential expression of ARHGAP35 indicates that the function of the protein may be different according to the tumour context and tissue type involved and not be the same in all malignancies.

The genetic alteration analysis found the mutations of ARHGAP35 to be relatively low frequency, typically less than 2% of the samples analysed. The high frequency of truncating mutations and amplifications, and lack of clear mutational hotspots, may suggest that ARHGAP35 is implicated in tumour-specific processes. In addition, the scattered mutation distribution in the lollipop plot and the distribution of mutations across functional domains of ARHGAP35 indicate that mutations in ARHGAP35 may affect cellular signalling pathways that have a role in cancer progression. There is also the potential for disruptions to regulatory signalling mechanisms due to the overlap of some mutations with phosphorylation sites. These observations are, however, based on bioinformatics studies and are meant to be suggestive of a mechanism rather than demonstrative of it.

Breast and cervical cancers revealed associations between ARHGAP35 alterations and poor clinical outcome in survival analyses, suggesting that ARHGAP35 status could be of prognostic relevance in specific tumour types. However, the current results cannot prove a causal relationship between ARHGAP35 changes and the disease progression or survival of the patients. Thus, the results of this study must be viewed as preliminary, and hypothesis generating.
Overall, the present study offers a detailed pan-cancer analysis of ARHGAP35 expression status, mutation profile and its potential clinical relevance. The findings indicate that ARHGAP35 might be involved in cancer-related processes in a context-dependent manner and may be a potential biomarker for future studies. Nevertheless, the present study lacks experimental, mechanistic and clinical validation studies to establish the function of ARHGAP35 and to establish its potential role in cancer diagnosis, prognosis and/or therapeutic development, and thus further studies are needed to confirm the findings of this study.

7. FUTURE PERSPECTIVES

Experimental validation of the observations made in the present bioinformatics analyses is needed for future studies to better understand the context-dependent role of ARHGAP35 in different types of cancer. *In vitro* cell culture-based functional studies, patient-derived models and *in vivo* animal models would be useful to elucidate the direct involvement of ARHGAP35 changes in tumour initiation, progression, migration and metastasis. Special emphasis needs to be placed on the investigation of the biological significance of mutations found in the RhoGAP domain and whether these mutations have a role in Rho GTPase mediated signalling pathways. Besides, research investigating post-translational modifications

such as the phosphorylation-based regulatory mechanism may also yield additional clues to the molecular regulation of ARHGAP35 and its downstream consequences in cancer-related signalling networks.
Important unresolved questions that should be explored in future studies include the need to include multi-cohort validation studies, establish standardised analytical pipelines, and increase the number of matched tumour-normal samples for large scale public data sets. Combining datasets from these two research areas of disease progression and therapeutic intervention could provide further insights into the dynamic role of ARHGAP35 in disease progression and therapeutic intervention. Further, integration of proteomic and transcriptomic data can facilitate identification of interacting partners, pathway associations and tumour-specific regulatory networks associated with ARHGAP35.
Clinically, the hypothesized relationship between ARHGAP35 changes and patient outcome in breast and cervical cancer suggests the need for further studies in independent prospective cohorts. However, its potential as a prognosis or biomarker should be taken with a pinch of salt until further large scale clinical and functional studies are done. There is potential for combination of ARHGAP35 signatures with other molecular and clinicopathological markers to enhance patient stratification and predictive modelling. Lastly, while direct targeting of ARHGAP35 may be difficult because of its proposed context-dependent and potentially tumour-suppressive activities, future research may investigate whether modulation of downstream signalling pathways that are downstream of ARHGAP35 dysregulation may provide therapeutic opportunities.

Author's contributions: Orchita Chakraborty conceptualised the study, did literature survey, carried out bioinformatics analysis, analysed the data, wrote and corrected the manuscript.

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