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ASB9 Across Human Cancers: A Comprehensive Pan-Cancer Bioinformatics Analysis

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ABSTRACT

ASB9 (ankyrin repeat and SOCS box containing 9) is a protein-coding gene that is associated with protein regulation, protein turnover, and creatine kinase activity. Its biological roles have been studied, but the landscape of genomic alteration in various cancers in humans is not fully characterized. In this study, we analyzed the genomic alterations, mutation profile, expression profile and the potential prognostic association of ASB9 in the publicly available cancer genomics datasets provided by cBioPortal and GEPIA2. In total, 148,790 patients and 156,010 samples from 12 datasets were analysed in this pan-cancer analysis. Only a small number of patients and samples were identified with detectable ASB9 alterations (212 in total, 215 samples) of the total of both patient and sample sets combined. The mutations identified were missense mutations, truncating mutations, amplifications, and deep deletions, which varied in prevalence by cancer type. A search for mutations identified substitutions at varying positions in the ASB9 protein sequence, with no particular hotspot apparent. The analysis of gene expression also showed variations in the expression of ASB9 between tumor and normal tissues in the examined cancer types. The overall survival analysis reported here did not find statistically significant differences between ASB9-altered and unaltered groups in the pan-cancer, colorectal cancer and melanoma analyses. The results offer a descriptive picture of the genomic changes and expression of ASB9 across human cancers and will aid in future studies of the biological role of ASB9.

Keywords: ASB9, Pan-cancer, Genomic alterations, Somatic mutations, cBioPortal, Colorectal cancer, Melanoma, Renal cancer

1. INTRODUCTION

The proper maintenance of protein homeostasis is crucial for the normal functions of cells. Involves the synthesis, folding, alteration and degradation of proteins to ensure the proper amounts of proteins are present in the cell. Ubiquitination is one of the key mechanisms of proteins regulation; it consists of attaching a small protein called ubiquitin to a target protein. The alteration may affect the stability, activity, localization and degradation of the protein of interest. Ubiquitination requires a series of enzymes, including E1, E2 and E3 enzymes. Of these, E3 ubiquitin ligases are key to engaging particular target proteins and to the transfer of ubiquitin. During this process, cells control the exercise of their proteins and various vital pathways of the signaling system (Sahasrabuddhe & Elenitoba-Johnson, 2014; Senft et al., 2017).

Several E3 ubiquitin ligase complexes are based on the cullin proteins. Cullin-5 is a protein which serves as a scaffold for the assembly of certain ubiquitin ligase complexes. These complexes are involved in the recognition of specific target proteins and control their stability by ubiquitination. Proteins belong to the ankyrin repeat and SOCS box (ASB) family which act as a substrate-specific component of the Cullin-5-dependent ubiquitin ligase complexes. The Ankyrin repeats are structural domains required for protein-protein interactions and the SOCS box is a protein domain that interacts with other components of ubiquitin ligase complexes. These structural properties enable the ASB family proteins to play a part in the regulation of particular cellular proteins.

The protein-coding gene, ASB9 (ankyrin repeat and SOCS box containing 9), belongs to the ASB family of proteins. It encodes a protein with ankyrin repeats and a SOCS box that are involved in protein interactions and regulating protein stability. There are previous studies that have associated ASB9 with regulation of creatine kinase and protein turnover (Debrincat et al., 2007; Thomas et al., 2013; Balasubramaniam et al., 2015). Creatine Kinase is an enzyme that helps to maintain cellular energy balance by removing or adding phosphate groups between creatine and ATP. The association of ASB9 with this enzyme suggests that the enzyme is involved in cell metabolic regulation. An understanding of the role of ASB9 in various cancer types, however, is still not fully understood.

Protein homeostasis is crucial to normal cell growth, survival and reaction to cellular stress. Cancer-related alterations in protein degradation and ubiquitin mediated signaling may impact many processes involved in cancer such as cellular proliferation, apoptosis, differentiation, and DNA damage responses. While many cancer researches concentrate on the most frequently mutated oncogenes and tumour suppressor genes, less frequently altered genes can also give clues on the molecular diversity of tumours. The frequency of genomic alterations does not alone provide clues about the importance of a gene. Biological relevance may also be due to changes in gene expression, protein stability or cellular regulatory pathways. In this context, ASB9 is interesting due to its reported role in protein regulation and cellular homeostasis. It is important to note, however, that its rather low prevalence of genomic alterations in most types of cancer should be taken with a pinch of a salt and cannot be interpreted as a cancer driver role solely based on its prevalence in the genome.

Bioinformatics analysis of cancer genomes from different cancers can be done using pan-cancer approach, which involves investigating the genomic alterations, patterns of mutations, gene expression and associations with clinical outcomes in multiple cancers through publicly available cancer genome data sets. These analyses can be used to investigate molecular patterns in various tumor populations and can be used to prioritise potential areas for further investigation. Variations in the cohorts, methods of genomic profiling, sample sizes, and clinical annotations, however, may affect the results. Therefore, it is important to interpret the results of pan-cancer analyses with care, especially when analysing different cancer types with varying numbers of cases.

Although ASB9 has been reported to be involved in protein regulation and cellular homeostasis, little is known about the genomic landscape of alterations in ASB9 in various human cancers. Data on the distribution of ASB9 mutations, alterations in copy number, expression patterns, and potential links to

patient survival is scarce. In-depth analysis of these characteristics may give a more comprehensive view of the ASB9 variation in tumour types and pinpoint areas in need of molecular and clinical study.

In the present study, we analyse the alterations of the ASB9 gene across publicly available cancer-genomics data, its somatic mutation patterns, its gene expression profile and its associations with survival outcomes. Cancer-specific analysis of selected cohorts, such as colorectal, melanoma, and renal cancer patients, is presented to provide deeper characterisation of ASB9 alterations. The findings offer a descriptive summary of ASB9 in human cancers and suggest avenues for further research of the biological and clinical importance of ASB9.

2. MATERIALS AND METHODS

2.1 Data Access

The Cancer Genome Atlas (TCGA) data was obtained from cBioPortal for Cancer Genomics (https://www.cbioportal.org/) and Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia2.cancer-pku.cn/#index). Using cBioPortal, genomic alterations, somatic mutations, copy number alterations, and clinical survival data were analyzed for ASB9 within selected cancer genomics studies. The gene expression data from The Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) project was used to investigate ASB9 gene expression in tumor and normal tissue groups with GEPIA2. The platforms offered free data on the genome and expression profiles of ASB9 in various cancer types (Tang et al., 2019).

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 ASB9 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. Studies lacking full genomic and/or clinical information are not included in the analysis.

A total of 12 pan-cancer analyses (148,790 patients and 156,010 samples) were included for the pan-cancer analysis. The datasets selected were: 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); and Tumors with TRK Fusions (MSK, Clin Cancer Res 2020).

The datasets selected were variable with respect to the composition of cohorts, sequencing methods, clinical features and genomic profiling coverage. The number of patients and samples listed is the sum of those in the studies selected and may not correspond to a number of equivalent people. The results were interpreted with regard to possible differences in cohort representation and possible overlap of datasets.

Forty lung cancer studies consisting of 15820 samples from 13158 patients and 33 non-small cell lung cancer studies with 15221 samples from 12765 patients were chosen based on the same inclusion criteria as above. Where a supplied ASB9 dataset had dedicated cohorts, additional cancer-specific analyses were performed, such as colorectal cancer, melanoma, and renal cancer.

Alteration frequencies were interpreted in relation to the evaluable samples within each individual cohort. Differences in sample size, sequencing approaches, and clinical annotation were considered when comparing alteration frequencies across cancer types. The analyses were treated as exploratory, and the combined dataset was not assumed to represent a single homogeneous patient population.

2.3 Gene Expression Analysis

The Gene Expression Profiling Interactive Analysis 2 (GEPIA2) web server (http://gepia2.cancer-pku.cn/) was used for gene expression analysis for ASB9, which uses the RNA sequencing data from The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) projects. The gene symbol "ASB9" was used to retrieve its expression profile across the available cancer types. The level of ASB9 transcripts in the tumour tissue was analysed and compared with that of normal tissue to determine if the abundance of ASB9 transcripts differs between the two. Graphical outputs of GEPIA2 were used for visualization of the expression profiles. Comparisons were made based on the data sets and the statistics that the platform provided.

2.4 OncoPrint Analysis

The genomic alteration profile of ASB9 was investigated and visualized in selected cancer types on the cBioPortal platform. The Symbol for the gene ‘ASB9’ was entered into the ‘Query by Gene’ function of cBioPortal, and the resulting genomic alteration information was retrieved from the selected cancer studies.

A visualisation of the genomic alteration profile was done with the aid of an OncoPrint, with individual columns representing tumour samples and the row representing ASB9. The platform-generated annotation and color coding were used to represent different classes of genomic alterations such as missense mutations, truncating mutations, splice-site alterations, amplifications, deep deletions, and multiple alterations. Samples with at least one detectable ASB9 genomic alteration were categorized as ‘altered’, and those without detectable genomic alterations as 'unaltered'. Unaltered group did not represent healthy individuals or normal tissue controls and was used as a genomic comparison group. The categories and frequencies of alterations were identified based on the annotation from the cBioPortal.

2.5 Analysis of Somatic Mutation Distribution

Somatic mutations in the ASB9 protein sequence were visualized in terms of their distribution across the gene sequence in cBioPortal. The mutations were displayed using a mutation distribution or lollipop plot of the position and abundance of mutations throughout the ASB9 protein sequence. Mutational alterations identified were classified based on the type of mutation, as annotated by the cBioPortal platform, such as missense mutations, truncating mutations, splice-site mutations, and in-frame mutations.

To determine the distribution of the positions of the detected mutations across the protein sequence and whether the recurrent mutation positions were found in the selected datasets, the positions and types of the detected mutations were analysed. The mutation plots were used to describe the observed mutation patterns. A mutation at a given position was not regarded as evidence of functional significance unless experiments confirmed it (Cerami et al., 2012).

2.6 Analysis of Survival Prognosis

Survival analysis was performed using the Comparison/Survival module of cBioPortal to examine clinical outcomes in patients with and without detectable ASB9 genomic alterations. Based on the status of genomic alterations, patients were grouped into altered and unaltered.

Survival curves were used to depict overall survival and other available survival outcomes and were constructed using the method of Kaplan–Meier. Overall survival was calculated using the clinical end point listed in the source data set. For overall survival, an event was defined as a recorded death in the follow-up period. Patients who were alive at the last clinical annotated follow-up were considered censored according to the clinical annotation of the source dataset.

Differences between survival distributions were evaluated using the log-rank test. A p-value below 0.05 was used as the nominal threshold for statistical significance. If adjusted q-values were available, they were presented separately from unadjusted p-values. When interpreting comparisons of survival, the number of patients, altered cases, and recorded events were taken into account as well as follow-up information. Analyses on a smaller size of altered groups or small number of recorded events were interpreted with great care, due to the possible low statistical power and instability of these analyses.

The statistical significance was considered as proof of the difference between the survival distributions within the cohort analysed and was not taken as evidence of clinical prognostic validity.

2.7 Genetic Alteration Analysis

The Cancer Types Summary module of the cBioPortal was used to quantify and profile genomic alterations in ASB9 in cancer types. Mutations, gene amplifications, and deep deletions were identified.

Alteration frequencies were obtained from the cBioPortal platform and represent the percentage of samples with detectable ASB9 alterations within each cohort. The platform-generated statistical summary and graphical output was used to describe the differences in alteration frequency between cancer types.

The rate of alteration observed was interpreted according to the number of evaluable samples and the information on the genome that was available in each case. Due to the difference in sequencing

approaches, populations studied, and sample size, comparisons among cohorts were therefore described.

3. RESULTS

3.1 Pan-Cancer Analysis of ASB9

The genomic alteration profile of ASB9 was first studied in a large pan-cancer cohort of 156,010 samples from 148,790 patients from 12 studies. The OncoPrint showed that the ASB9 genomic alterations were found across a number of cancer types and comprised missense mutations, truncating mutations, amplifications and deep deletions (Figure 1). Modifications were noted in a majority of the studies examined but were not present in all studies, and the incidence of the modifications differed between cancer types (The Cancer Genome Atlas Network 2012; The Cancer Genome Atlas Research Network 2013; 2014).

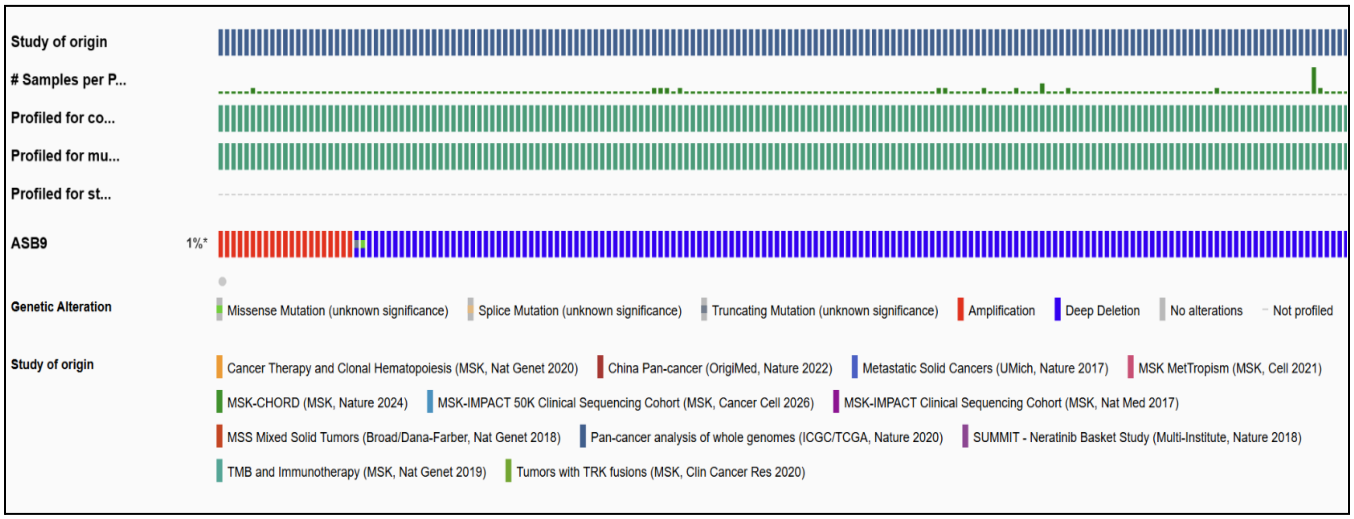


Fig. 1. OncoPrint analysis. The OncoPrint illustrates the distribution of detectable ASB9 genomic alterations across the selected cancer-genomics datasets.

The total queries yielded 212 patients and 215 samples that contained detectable ASB9 alterations (as illustrated in Figure 2). These made up less than 1% of the total population queried. These frequencies of alterations differed by cancer type, with the highest frequency being approximately 21% among patients with lung cancer. Other cancers, including esophagogastric, endometrial, ovarian, hepatobiliary, germ-cell, head-and-neck, bone, pancreatic, and melanoma cancers, also experienced a decrease in frequencies.

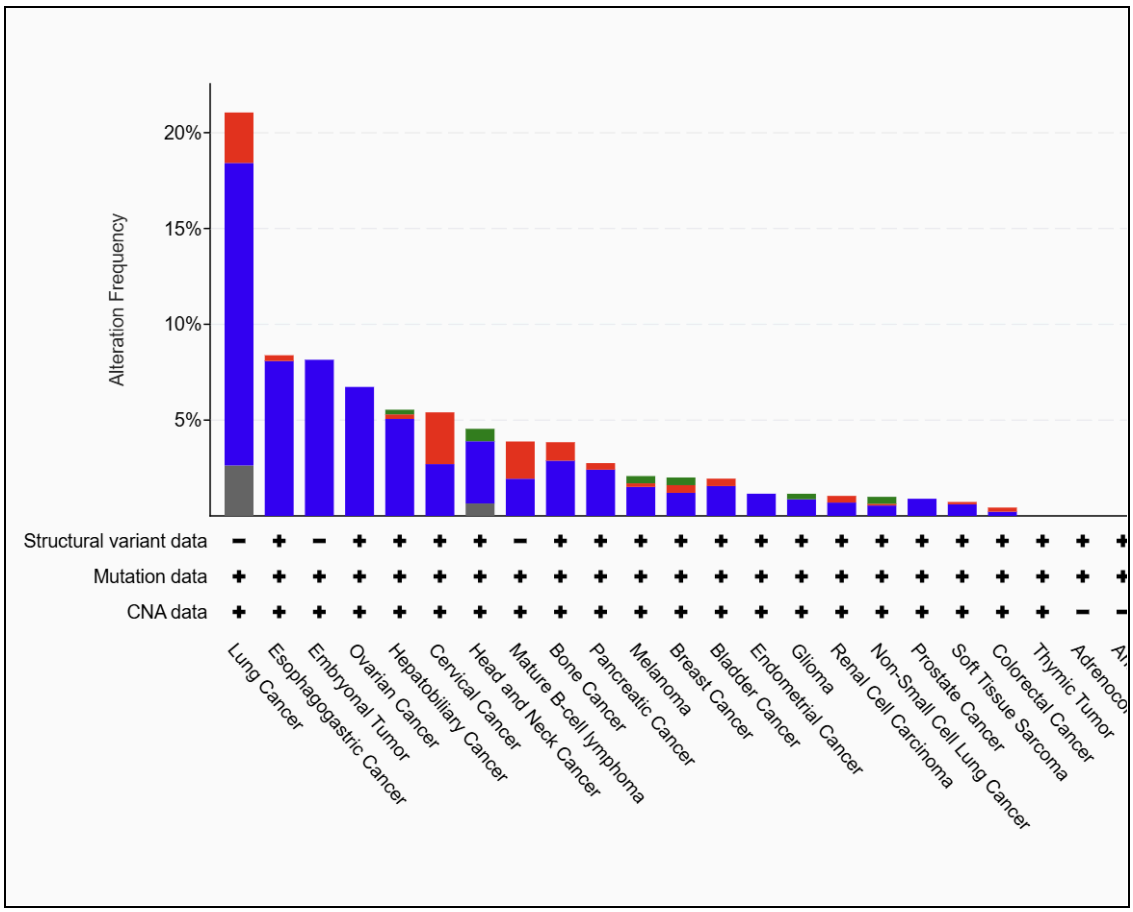


Fig. 2. Genetic alteration analysis. The bar graph indicates the alteration frequency of ASB9 in different cancer types.

The somatic mutation distribution of ASB9 was plotted through its 294-amino-acid protein sequence (Figure 3). The mutation annotation showed no obvious hotspot of mutations throughout the sequence of ASB9, but rather multiple amino acid substitutions scattered throughout the sequence. One highlighted alteration was R143W in the ankyrin-repeat region, while other mutations were found throughout the protein.

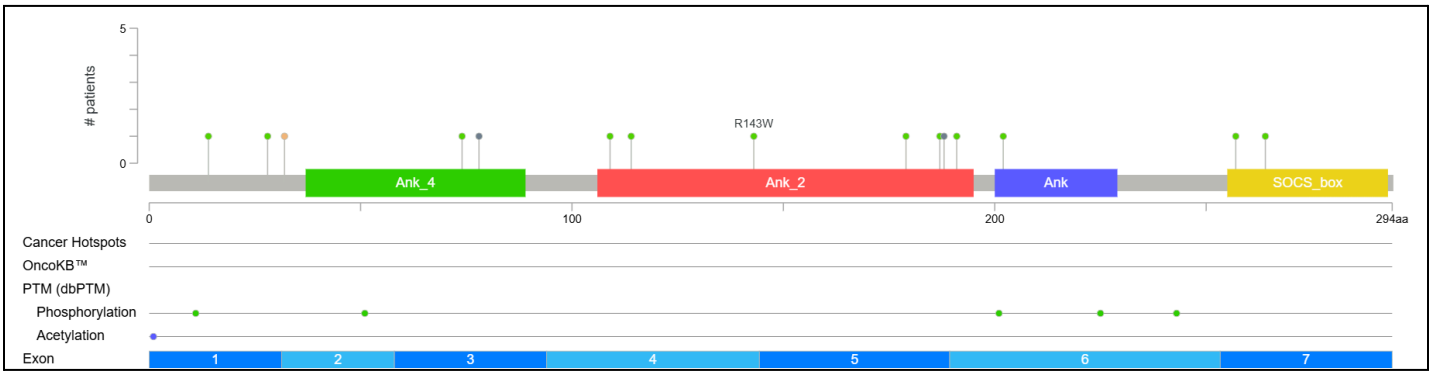


Fig. 3. Distribution of ASB9 somatic mutations and protein domains. The ASB9 protein consists of 294 amino acids and contains ankyrin-repeat regions and a SOCS box.

The overall survival analysis was performed on a group of 530 patients: 25 patients who were altered with ASB9 and 505 patients who were not altered with ASB9. In the altered group, 13 events were recorded and in the unaltered group 214 events were recorded. Median overall survival (OS) was 28.62 months in the altered group and 37.04 months in the unaltered group. There was no statistically significant difference between the two groups using the log-rank test (p = 0.491) (Figure 4).

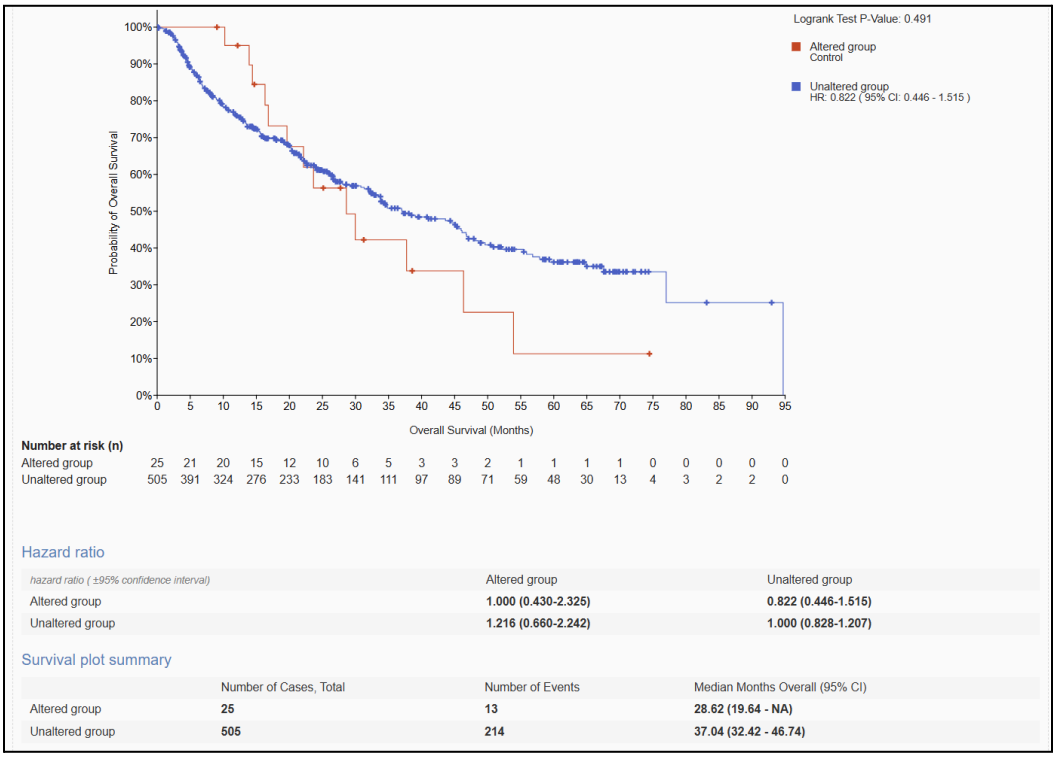


Fig. 4 Kaplan–Meier overall survival analysis. ASB9-altered and unaltered patients in the pan-cancer cohort.

3.2 ASB9 Gene Expression Across Cancers

GEPIA2 was used to analyze the expression profile of ASB9 by comparing RNA expression levels between tumor and normal tissue groups across the available cancer types (Figure 5). The expression of ASB9 differed between the tumor and normal tissue groups with direction and magnitude of the differences varying by cancer type. The expression profiles showed differences in the level of expression of the ASB9 transcripts in the different tumor populations analyzed.

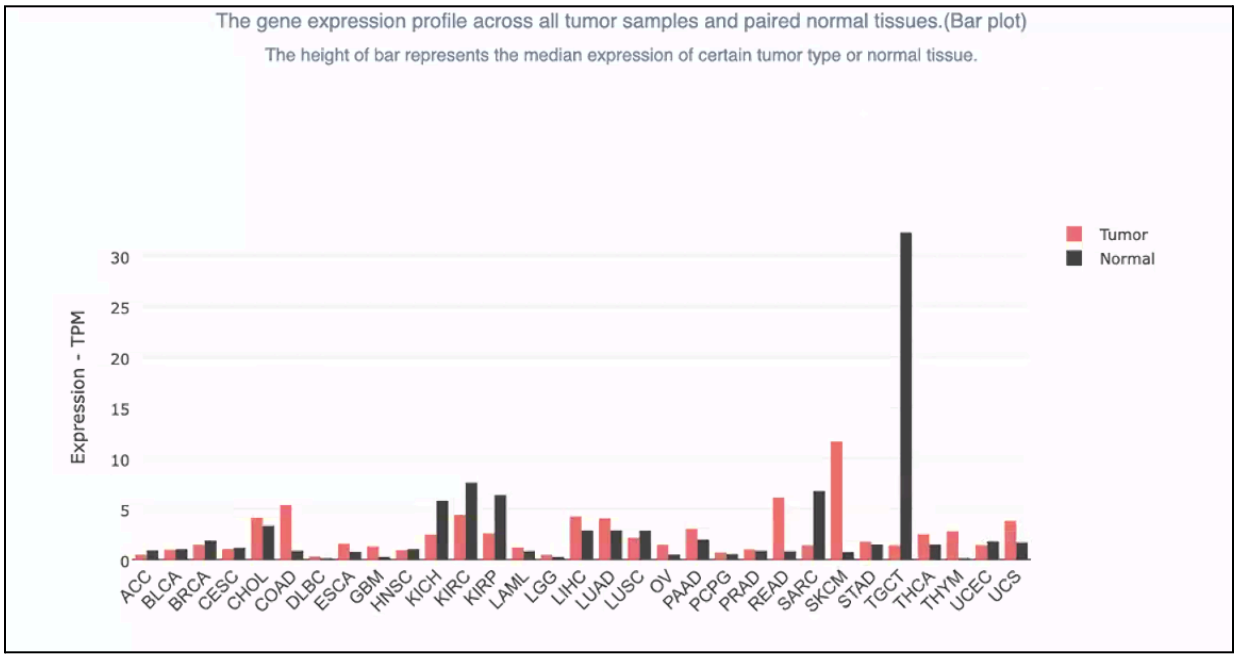


Fig. 5. Gene expression analysis. The bar graph indicates ASB9 expression levels in tumor tissues and their corresponding normal tissues.

3.3 Role of ASB9 in Colorectal Cancer

The genomic alteration status of ASB9 was subsequently analyzed in 20 colorectal cancer cohorts with a total of 7,558 samples from 7,308 patients. Fifteen patients were identified by the OncoPrint analysis in the selected datasets with detectable ASB9 alterations (Figure 6). Different classes of genomic alterations were observed in each of the colorectal cancer cohorts.

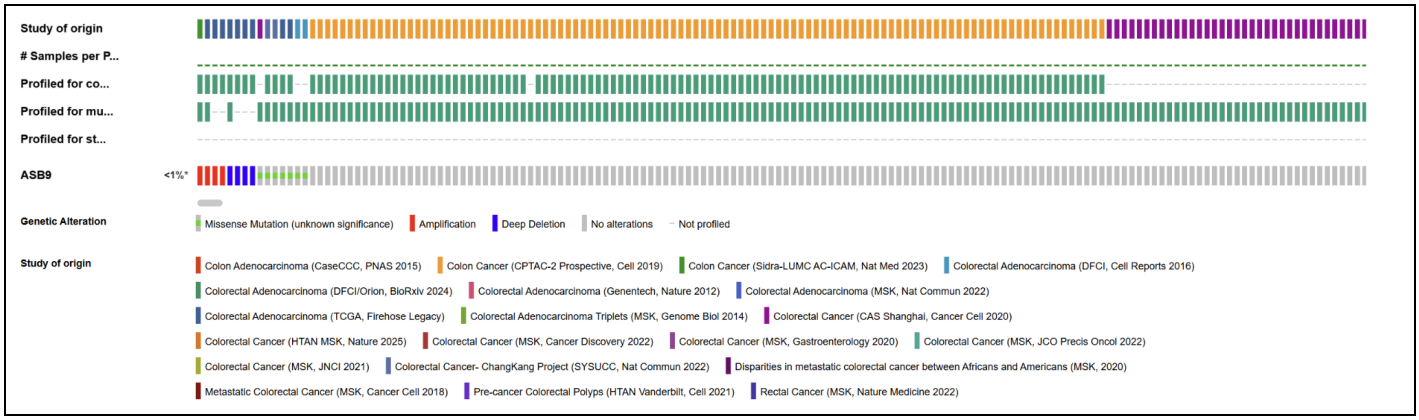


Fig. 6. OncoPrint analysis of ASB9 in colorectal cancer. Genomic alterations in ASB9 across multiple colorectal-cancer datasets.

Further genetic alteration analysis showed that ASB9 alterations were present at relatively low frequencies across the colorectal-cancer cohorts. The highest visible frequency was 1.45%, while other cohorts had lower frequencies.

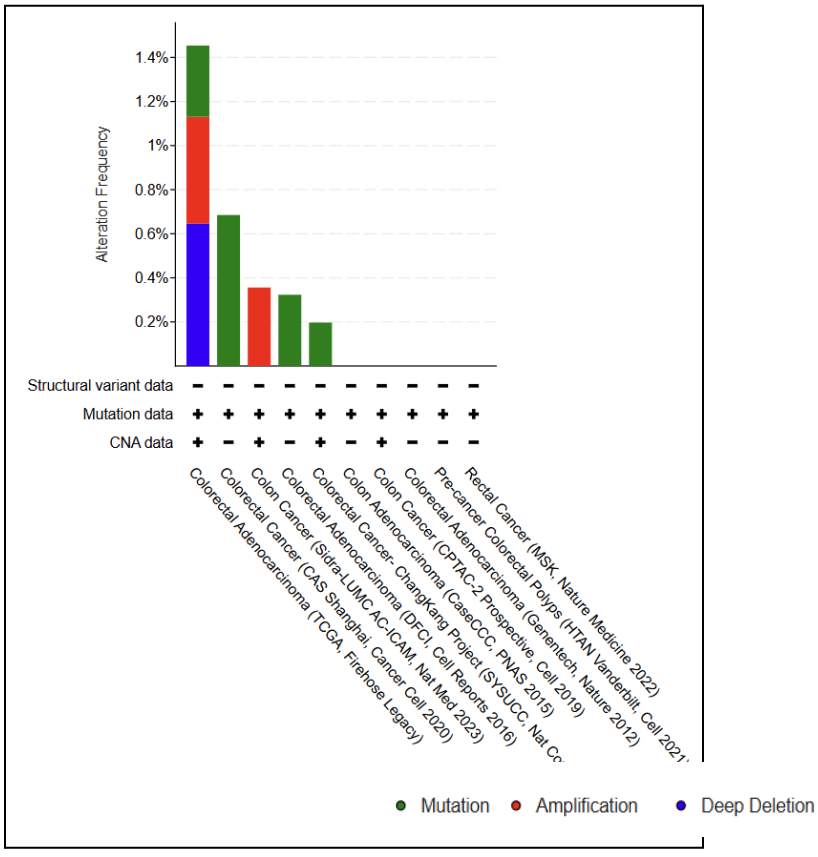


Fig. 7. Genetic alteration analysis of ASB9 in colorectal cancer. The bar graph indicates the alteration frequency of ASB9 across colorectal cancer cohorts.

Somatic mutation analysis revealed that the mutation in the ASB9 protein was G168D. The mutation was in the ankyrin-repeat domain of the protein.

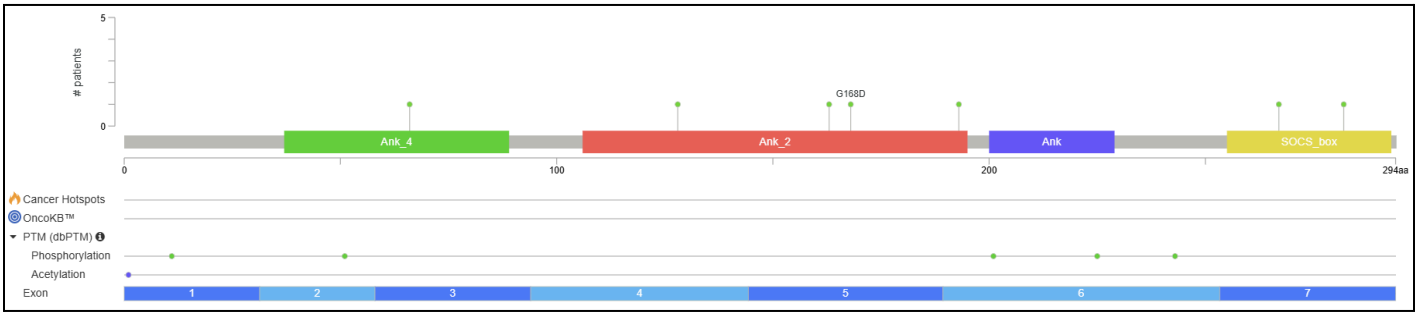
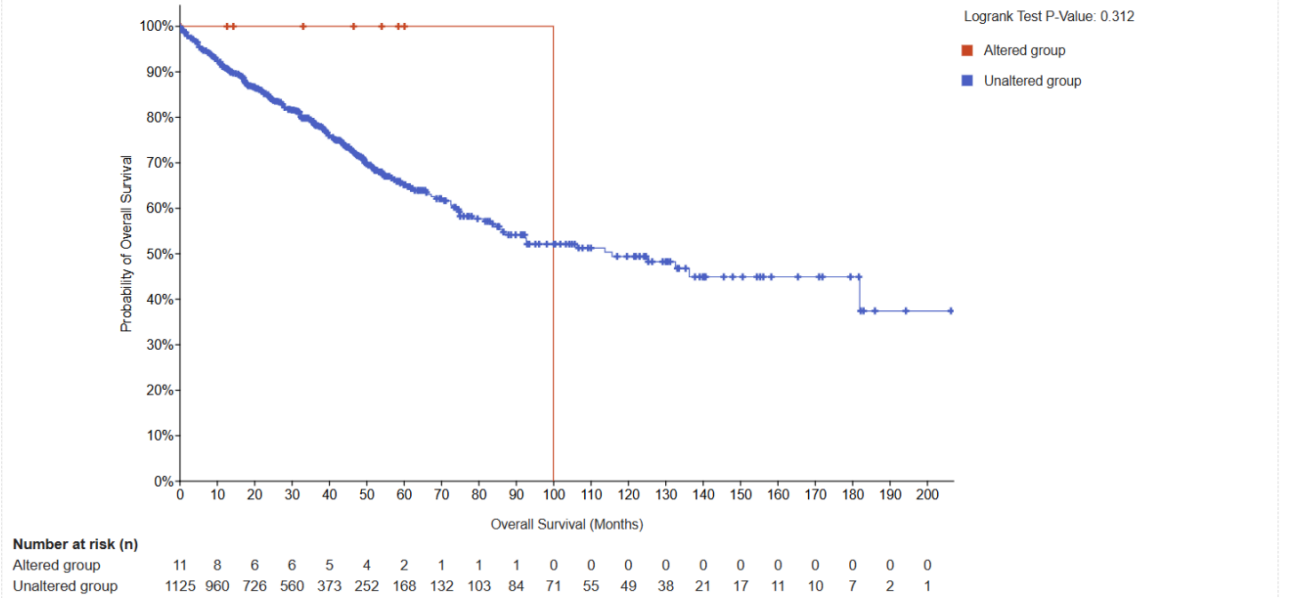


Fig. 8. Distribution of ASB9 mutations in colorectal cancer. The lollipop plot illustrates the distribution of somatic mutations in ASB9 along the protein sequence across colorectal cancer samples

Survival analysis was carried out on patients with colorectal cancer based on the status of the ASB9 alteration. 1,379 patients were included for disease-free survival, with $p = 0.0383$ and $q = 0.0767$ reported by the platform. For overall survival, 1,136 patients were analyzed, with $p = 0.312$ and $q = 0.416$ (Figure 9). In overall survival, the ASB9-altered group consisted of 11 patients with one recorded event, and the median overall survival was approximately 99.93 months. The unaltered cohort comprised 1125 patients and 278 recorded events, with a median overall survival of around 115.62 months.



Survival plot summary

	Number of Cases, Total	Number of Events	Median Months Overall (95% CI)
Altered group	**11**	**1**	**99.93**
Unaltered group	**1125**	**278**	**115.62 (86.21 - NA)**

Fig. 9. Analysis of survival prognosis. The Kaplan–Meier plot indicates the probability of overall survival in ASB9-altered and unaltered colorectal cancer groups.

3.4 Role of ASB9 in Melanoma

cBioPortal OncoPrint analysis was used to further investigate the ASB9 alterations in melanoma datasets. The OncoPrint indicated that alterations in ASB9 were relatively rare, with respect to the number of melanoma studies analyzed. Missense mutations and copy number loss were seen, but most samples did not have any detectable ASB9 alterations (Figure 10).

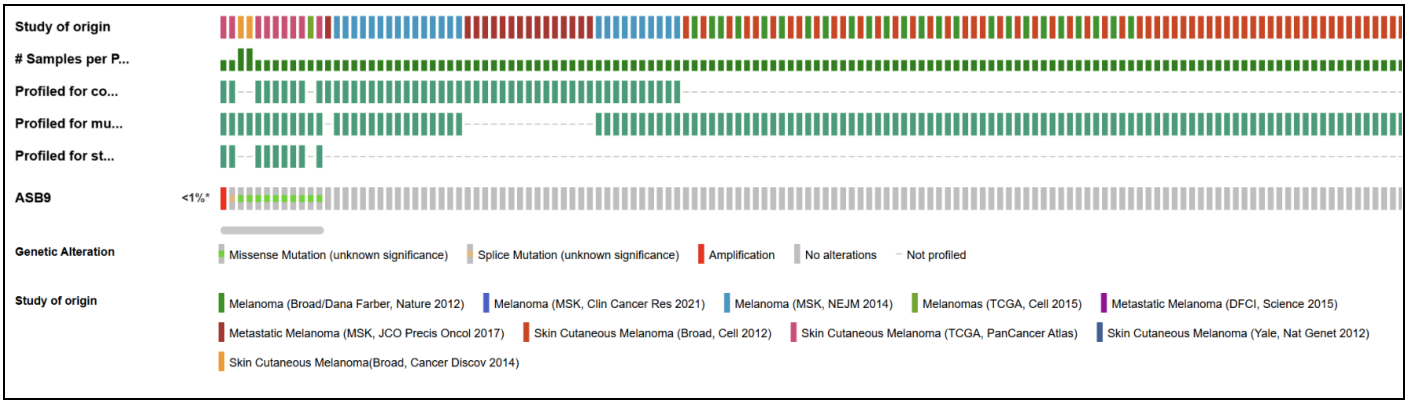


Fig. 10. OncoPrint analysis of ASB9 in Melanoma. Genomic alterations in ASB9 across melanoma datasets.

Within the melanoma Cancer Types Summary, ASB9 alteration frequency varied across cohorts (Figure 11). The alteration frequency ranged from about 4.3% in the highest cohort to 2.0% in another cohort, with other cohorts exhibiting frequencies of less than 1%.

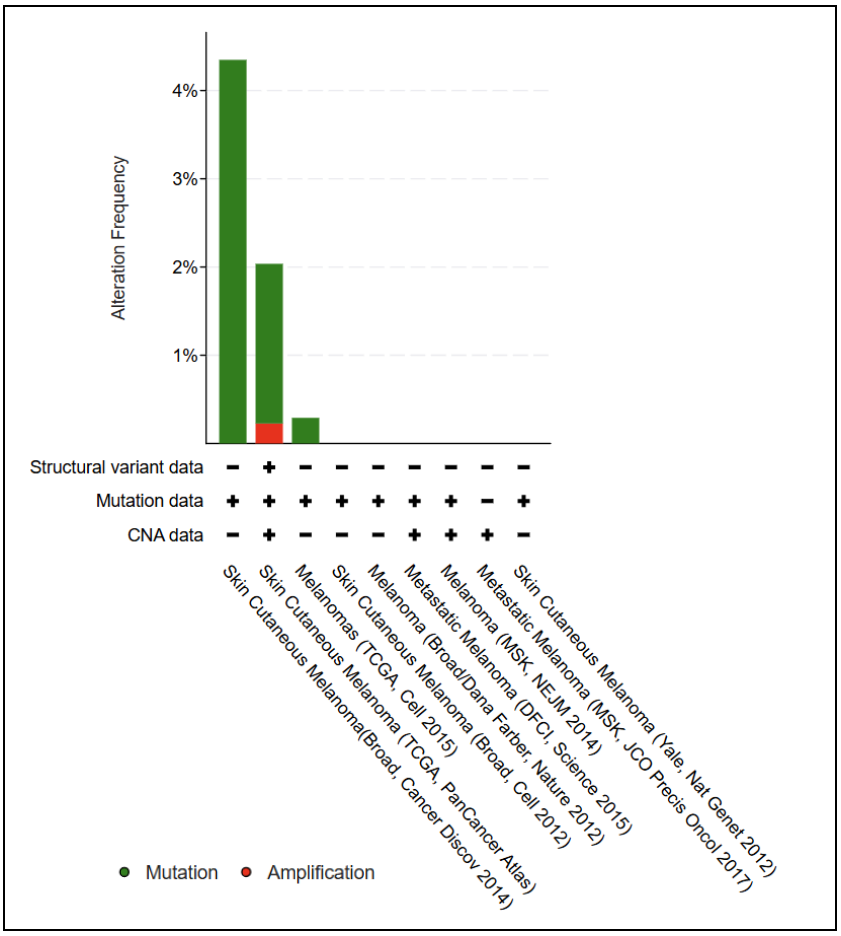


Fig. 11. Genetic alteration analysis of ASB9 in Melanoma. The bar graph indicates the alteration frequency of ASB9 across different melanoma cohorts.

A total of 13 ASB9 mutations were identified by the melanoma mutation analysis, 12 of which were missense mutations. The total somatic mutation rate was 0.6%. The P13T mutation was indicated in the mutation plot. Mutations were spread over the ASB9 protein with no apparent dominant recurrent hotspot.

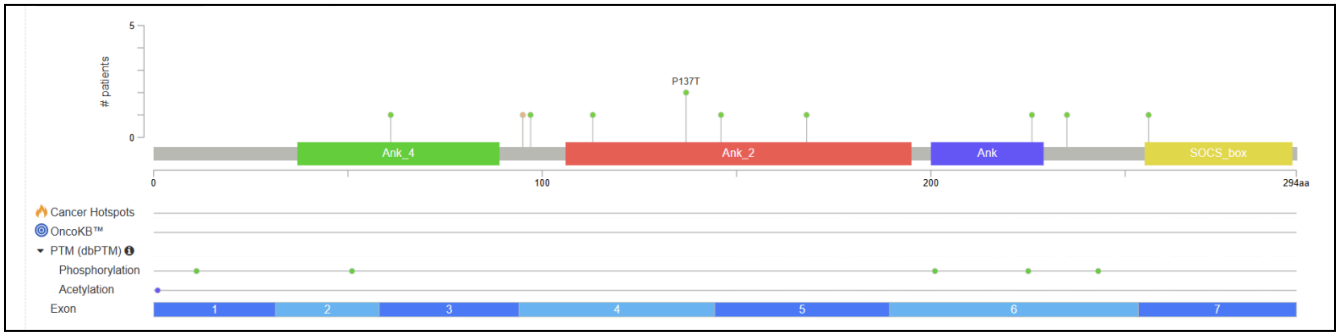
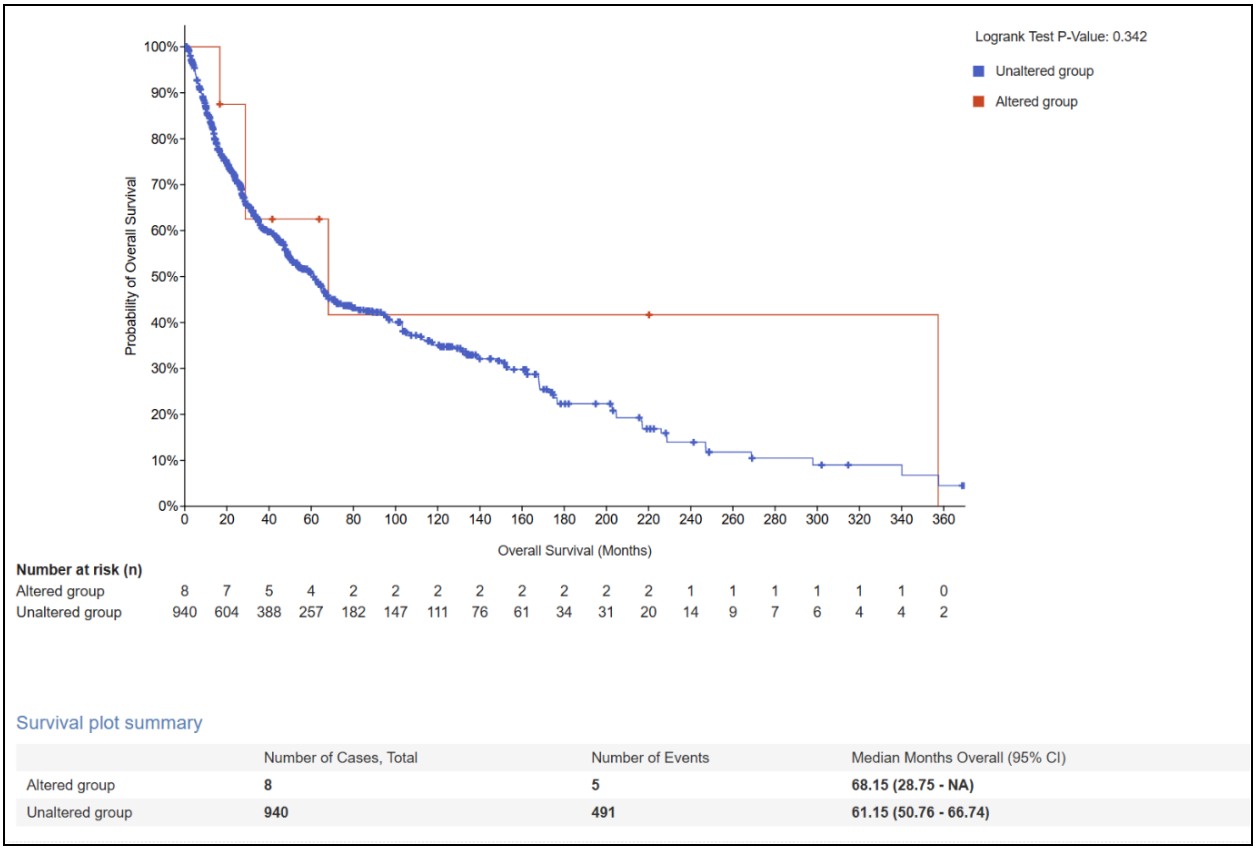


Fig. 12. Distribution of somatic ASB9 mutations in melanoma. P13T is a highlighted mutation.

Survival analysis was conducted in melanoma based on ASB9 alteration status. A progression-free survival analysis was performed with 428 patients, yielding p = 0.0440 and q = 0.132. Disease-specific survival was performed in 421 patients with p = 0.329 and q = 0.342, and overall survival in 948 patients with p = 0.342 and q = 0.342. Only 8 patients were included in the group of ASB9-altered patients (5 events), and the median overall survival was ~68.15 months. The unaltered group involved 491 events and 940 patients, whose median overall survival was ~61.15 months. There was no statistically significant difference in overall survival in the two groups despite the longer median survival in the altered group (log-rank p = 0.342).



Survival plot summary

	Number of Cases, Total	Number of Events	Median Months Overall (95% CI)
Altered group	**8**	**5**	**68.15 (28.75 - NA)**
Unaltered group	**940**	**491**	**61.15 (50.76 - 66.74)**

Fig. 13. Analysis of survival prognosis in Melanoma. The Kaplan–Meier plot indicates the probability of overall survival in ASB9-altered and unaltered Melanoma groups.

3.5 Role of ASB9 in Renal Cell Carcinoma

Only <1% of samples detected alterations; as in most other cohorts analyzed, they were only amplification events and did not contain missense, truncating, or deletion events (Figure 14). The frequency of alterations varied between the two histological subgroups , and only detectable ASB9 amplification (around 0.4%) was found in one subgroup, whereas no alterations were found in the other (Figure 15). This is reflected by the absence of any somatic mutations found over the entire ASB9 protein sequence in this cohort by mutation distribution analysis (Figure 16).

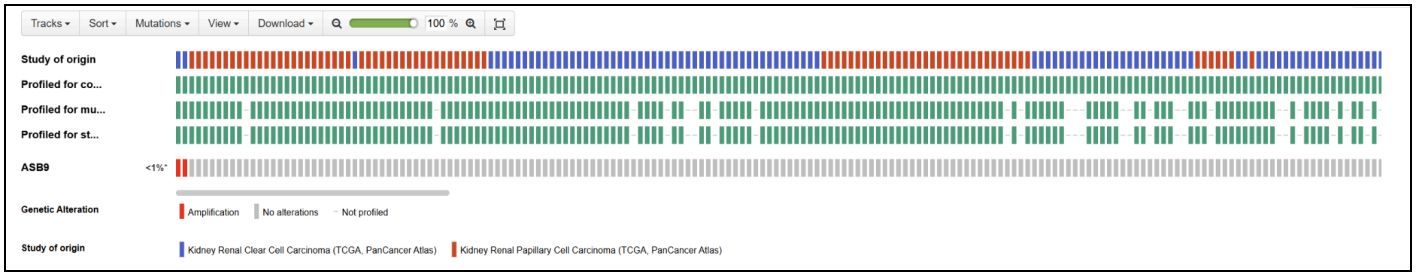


Fig. 14. OncoPrint analysis of ASB9 in renal cell carcinoma datasets.

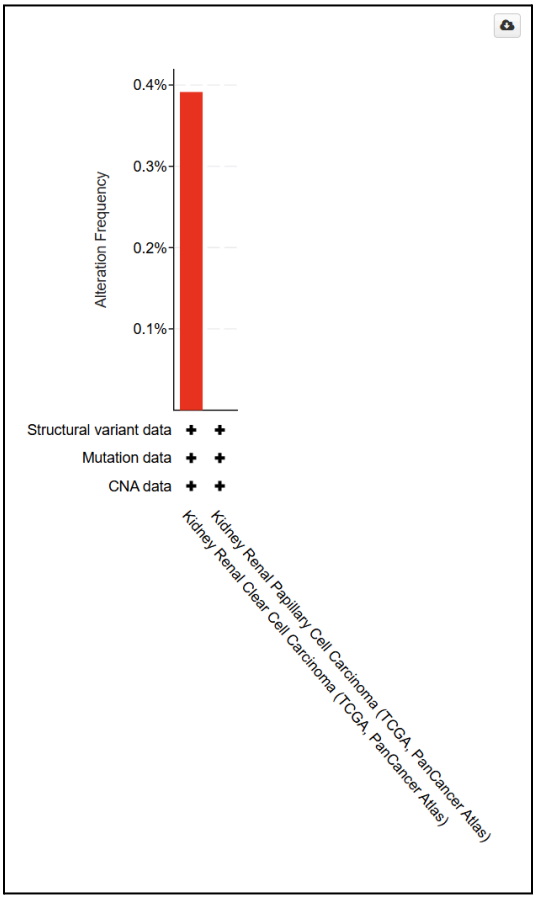


Fig. 15. Genetic alteration frequency of ASB9 in renal cell carcinoma

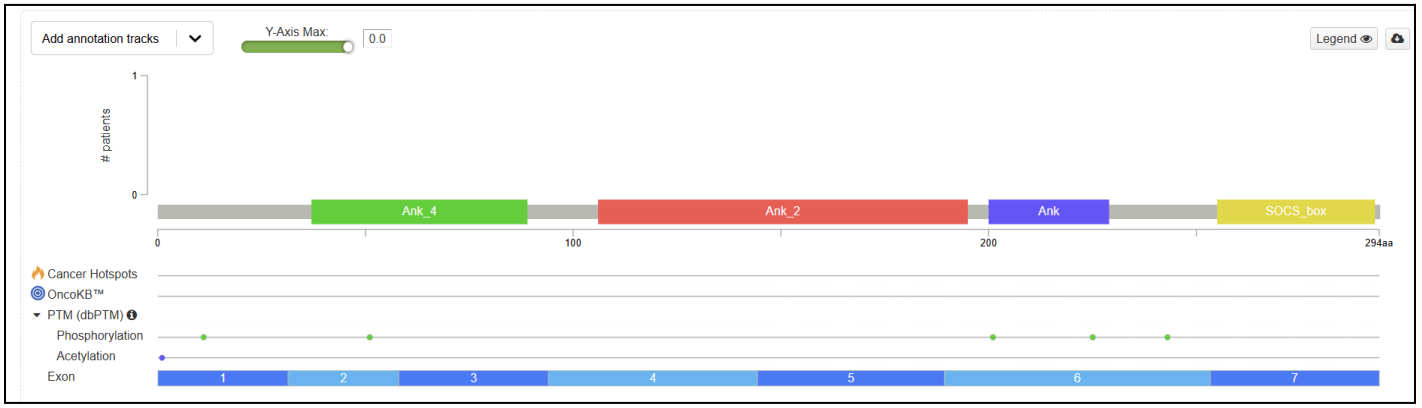


Fig. 16. Somatic mutation analysis of ASB9 in renal cell carcinoma

The Kaplan–Meier analysis showed that there was a significant difference between the ASB9-altered and unaltered groups (log-rank p-value = 1.54×10^{-14}; Figure 17). This discovery suggests that there is a statistical correlation between the status of ASB9 alteration and survival for the renal cell carcinoma cohort analyzed. This is, however, a preliminary finding since there were relatively few ASB9-altered cases, and it needs to be confirmed in other independent renal cancer patient cohorts.

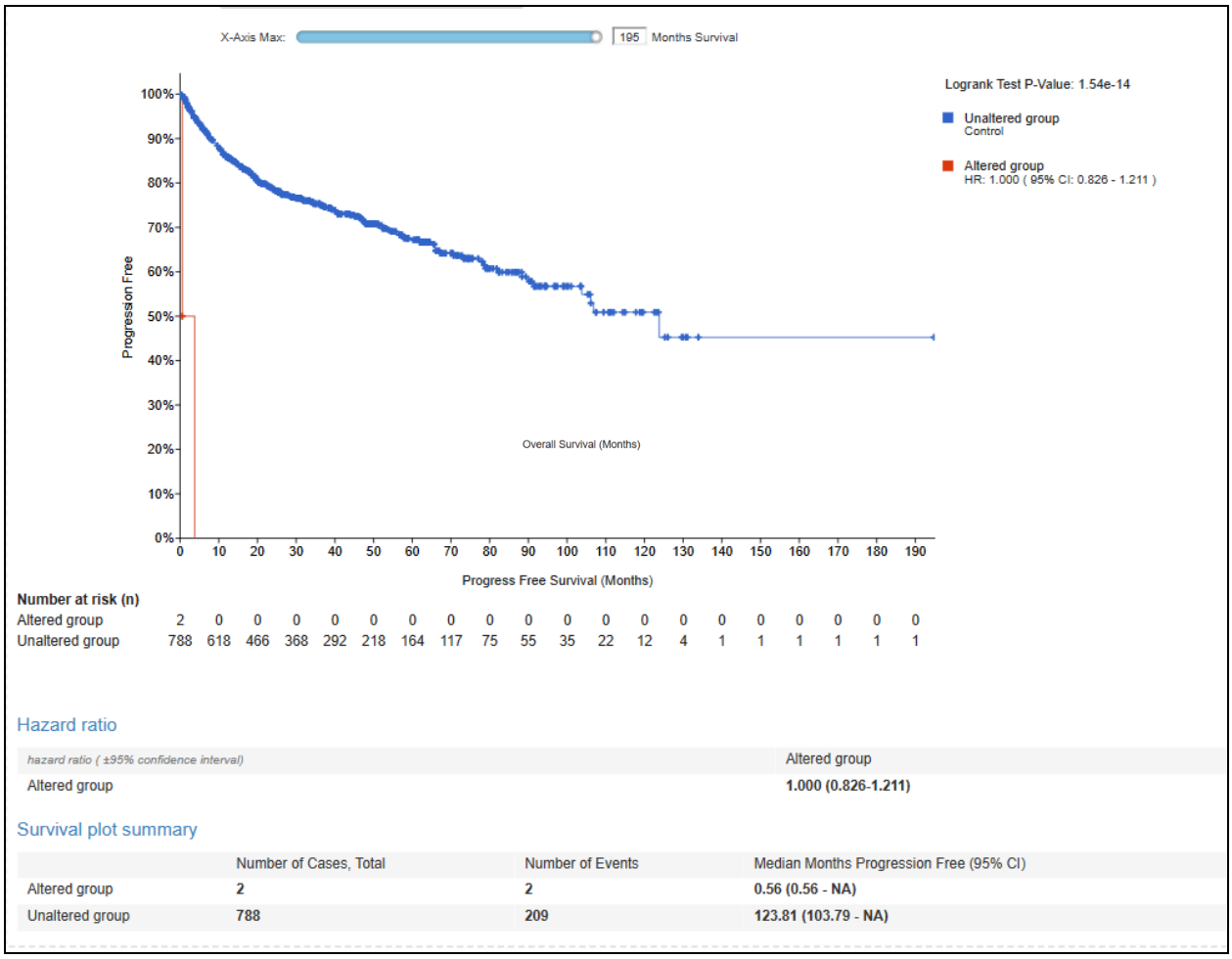


Fig. 17. Survival analysis of ASB9 in renal cell carcinoma

4. DISCUSSION

The ASB9 (Ankyrin Repeat and SOCS Box Containing 9) gene encodes a member of the ASB family of proteins and is a component of a substrate-recognition complex that is part of a Cullin-5-based E3 ubiquitin ligase. ASB9 is involved in protein recognition and ubiquitin-mediated degradation, especially the recognition and degradation of creatine kinase proteins, by its ankyrin-repeat domains and SOCS box. ASB9 has been demonstrated to interact with Brain-type creatine kinase and mitochondrial creatine kinase, where it may have a role in cellular energy control and protein homeostasis (Debrincat et al. 2007; Thomas et al. 2013). Metabolic reprogramming and protein degradation are key events during tumour progression, so it is biologically relevant to explore the expression and genomic features of ASB9 in various types of tumours.

ASB9 genomic events were not particularly frequent in the cancer types included in the analysis but this does not preclude biological significance as it is observed that these events are rare in the genome. ASB9 has been linked to protein turnover and creatine kinase regulation both of which are related to cellular protein and metabolic homeostasis. The current analysis offers a general picture of the distribution of ASB9 alterations and expression pattern among various human cancers. Observed changes in the genome do not necessarily mean that ASB9 is directly involved in the development or progression of tumors, however. It may play a role in processes other than detectable genomic changes, including changes in gene expression and protein regulation.

The ASB9 mutation profile was also varied, with mutations spread across the protein, and not clustered in a single dominant mutational hotspot. These mutations show variation in type and location of the reported genomic changes. The biological relevance of the mutations found in the various amino-acid positions, including those related to the ankyrin repeat, is evident in their relation to substrate recognition and protein-protein interaction. Structural investigations have shown that the ankyrin-repeat domain of ASB9 is required for binding to substrates of creatine kinase, and the SOCS box is required for the assembly of the ubiquitin ligase complex (Thomas et al. 2013). Thus, mutations in these functional regions may impact the ability to bind to the substrate, protein stability, or ubiquitination activity. However, the functional consequences of the observed ASB9 variants are still not known and need to be confirmed through experimentation.

The results in colorectal cancer are of particular significance because of previous data indicating a possible tumour-suppressive effect of ASB9. Mori (2010) has shown that decreased ASB9 expression correlated with increased invasive behaviour and worse clinical outcome in colorectal cancer. Based on the present findings, genomic alteration and altered gene expression should be interpreted separately, as a gene can be involved in the progression of tumours in a transcriptional or post-transcriptional manner without being frequently mutated. This may be responsible for the fact that ASB9 retains biological relevance in some cancers despite the fact that the genomic changes are relatively rare.

However, the renal cancer survival analysis needs special attention due to the limited number of cases with altered ASB reported in the original analysis. The log-rank test yielded a statistically significant result, but survival estimates may be unstable and not generalizable due to the small number of subjects in the altered group.

In summary, this pan-cancer analysis offers a descriptive picture of ASB9 genomic alterations, patterns of mutations and expression in various human cancers. ASB9 changes were not seen in a high proportion of the analyzed cancers, but were distributed differently in the different cancer cohorts, reflecting the molecular diversity between the different cancers. The results suggest that ASB9 might play a role in protein regulation and cellular homeostasis, and further studies are warranted into the biological functions of ASB9, especially its role in protein regulation. In several cohorts, there were, however, relatively few patients with alterations, and the nature of the analyses of survival was exploratory, so it is not possible to draw any firm conclusions about its prognostic significance. Future studies combining independent clinical cohorts with functional validation are needed to establish the biological and potential clinical relevance of ASB9 in cancer.

5. LIMITATIONS

The present study has a few limitations. First, the analysis was limited to publicly available genomic and transcriptomic datasets that may have differences in patient characteristics, sample collection, sequencing methodology, and data processing. Secondly, the frequency of alterations in ASB9 in the cancers analyzed was low, leading to small numbers of patients with altered in some cohorts, compromising statistical power of some analyses, especially those involving survival comparisons. Due to the small number of samples used for these analyses, and the risk of chance associations, the results should be interpreted with caution for potentially being unstable estimates. Hence the observed survival patterns are descriptive and are not considered to be a unique prognosis variable for ASB9. These results need to be confirmed in larger and well powered cohorts. Thirdly, the study was retrospective and involved bioinformatics analysis with no experimental confirmation of the expression of ASB9 and/or the functional effects of the identified mutations, including R143W, G168D, and P13T. In addition, the available datasets limited longitudinal and treatment-specific information and could not be used to determine whether ASB9 alterations impact treatment response or disease progression. The associations observed may also be influenced by confounding clinical and molecular factors which were only partially controlled. However, more studies in independent patient groups, functional characterization, and experimental validation are needed to prove the biological significance of ASB9 alterations and their possible clinical relevance in cancer.

6. CONCLUSION

The pattern and possible prognostic role of ASB9 were systematically explored in available publicly released cancer datasets. Pan-cancer analysis showed that, in general, alterations in ASB9 are not frequent, but the frequency of alterations did differ by cancer type, with the lung cancer category showing the highest frequency of alterations. There were also significant differences in the expression of ASB9 between normal and tumor tissues, including between tissues that have different biological functions.

The genomic analysis revealed a variety of ASB9 alterations, including amplification, missense, splice, truncating, and deletion events, which were all heterogeneous. Mutations were found throughout the ASB9 protein and included mutations in or close to the ankyrin-repeat regions. Cancer-specific analyses revealed changes in colorectal cancer, melanoma, and renal cancer, but with low frequencies in most cohorts.

Even though the analyses for pan-cancer, colorectal cancer, and melanoma did not reveal any overall survival differences, the renal cancer analysis revealed a significant association between the alteration status of ASB9 and survival. However, due to the limited number of ASB9 altered cases in some groups, some of these comparisons are of limited statistical power and therefore represent exploratory results. So, the present findings cannot be used to validate ASB9 as a prognostic biomarker. In summary, this study aids in the characterization of ASB9 in the pan-cancer context and serves as a groundwork for additional studies on the biological relevance of ASB9. The genomic and expression patterns observed in the small clinical cohorts and experimental studies are yet to be validated in larger cohorts and experiments and the relevance to cancer biology and clinical outcomes is yet to be determined.

7. FUTURE PERSPECTIVES

In vitro and in vivo models of cancers in which an alteration or differential expression of ASB9 was observed should be used to further investigate the observed genomic alterations, mutations, and expression patterns of ASB9. These mutations, such as R143W, G168D, and P13T, should be particularly investigated for their biological significance and their possible impact on ASB9 protein stability, substrate recognition, ubiquitination, and cellular energy metabolism by functional studies.

Future studies should also involve independent multi-cohort validation, increased numbers of matched tumor-normal samples, and incorporation of genomic, transcriptomic, and proteomic data. Further studies are required to investigate whether there is a correlation between expression levels of ASB9, or differences in the genome, and molecular subtypes, drug sensitivity, or disease progression. Furthermore, the investigation of ASB9 in conjunction with other well-known molecular and clinicopathological markers might facilitate patient stratification and give additional hints to the diagnostic, prognostic, or therapeutic value of ASB9 in cancer.

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