# Somatic Mutations in Collagens are Associated with a Distinct Tumor Environment and Overall Survival in Gastric Cancer

ALEXANDER BRODSKY ( ✉ alex_brodsky@brown.edu )

https://orcid.org/0000-0001-7357-5153

**Jay Khurana**
Brown University

**Kevin S Guo**
Brown University

**Elizabeth Y. Wu**
Rhode Island Hospital

**Dongfang Yang**
Rhode Island Hospital

**Ian Y. Wong**
Brown University

**Ece D. Gamsiz Uzun**
Rhode Island Hospital

**Murray B. Resnick**
Brown University

---

**Research article**

---

# Abstract

## Background

Gastric cancer is a heterogeneous disease with poorly understood genetic and microenvironmental factors. Mutations in collagen genes are associated with genetic diseases that compromise tissue integrity, but their role in tumor progression has not been extensively reported. In contrast, aberrant collagen expression has been long associated with malignant tumor growth, invasion, chemoresistance, and patient outcomes. We hypothesized that somatic mutations in collagens could functionally alter the tumor microenvironment, including the extracellular matrix.

## Methods

We used publicly available datasets including the Tumor Cancer Genome Atlas (TCGA) to interrogate somatic mutations in collagens in stomach adenocarcinomas. To demonstrate that collagens were significantly mutated above background mutation rates, we used a moderated Kolmogorov-Smirnov test along with combination analysis with a bootstrap approach to define the background. Association between mutations and clinicopathological features was evaluated by Fisher or chi-squared tests. Association with overall survival was assessed by Kaplan-Meier and the Cox-Proportional Hazards Model. Gene Set Enrichment Analysis was used to interrogate pathways. Immunohistochemistry and *in situ* hybridization tested expression of COL7A1 in stomach tumors.

## Results

In stomach adenocarcinomas, we identified individual collagen genes and sets of collagen genes harboring somatic mutations at a high frequency compared to background in both microsatellite stable, and microsatellite instable tumors in The Cancer Genome Atlas (TCGA). Many of the missense mutations resemble the same types of loss of function mutations in collagenopathies that disrupt tissue formation and destabilize cells providing guidance to interpret the somatic mutations. We identified combinations of somatic mutations in collagens associated with overall survival, with a distinctive tumor microenvironment marked by lower matrisome expression and immune cell signatures. Truncation mutations were strongly associated with improved outcomes suggesting that loss of expression of tumor cell secreted collagens have large impacts on tumor progression and treatment response.

## Conclusions

These observations highlight that many minor collagens, expressed in non-physiologically relevant conditions in tumors, secreted from tumor cells, harbor impactful somatic mutations in tumors, suggesting new approaches for classification and therapy development in stomach cancer. In sum, these

findings demonstrate how classification of tumors by collagen mutations identified strong links between specific genotypes and the tumor environment.

## Background

Collagens are the most abundant proteins in extracellular matrix and are critical components and regulators of the tumor microenvironment (1, 2). Increased collagen expression in many solid tumors has been associated with poor outcomes and resistance in multiple settings (3), likely through increased epithelial-to-mesenchymal transitions (EMT) and drug resistance (4). The 28 members of the collagen family are expressed by 43 genes and defined by the common triple helix motif. Collagens are classified into families including fibrillar collagens (i.e. Collagen type I, II, III, V, XI, XIV), network collagens (i.e. Collagen type IV), membrane (i.e. type XVII) and other (type VII, XXVIII) (5). Although most studies have focused on the most abundant collagen, collagen type I, and the major basement membrane collagen, type IV, there is increasing awareness of the role of many minor collagens in cancer such as types X and XI (6, 7). The breadth of mechanisms by which collagens mediate tumor progression is not yet understood and collagens could have context dependent functions in tumors (4). In fact, it is not always clear which cells even express collagen in tumors as both cancer and stroma cells are known to secrete collagens as indicated both by *in situ* hybridization studies (8, 9) and recent proteomic studies also suggest tumor cells secrete collagens (10). Much of the fibrillar collagen is thought to originate from fibroblasts in stomach adenocarcinoma (STAD) (4).

Worldwide, gastric cancer remains one of the top deadliest malignancies (11). Advanced gastric tumors are treated with surgery and chemotherapy with 5-year survival rates above 50% if the disease has not spread, and < 10% if metastasis has occurred (12). The connections between therapy outcomes, the stroma, and collagens remains uncertain in stomach cancer. Physical properties of collagen fibers have been associated with outcomes in gastric cancer (GC) and these observations are likely driven by the most abundant collagen, type I (13). Collagen type I expression has been associated with metastasis in early onset gastric cancer (14).

Towards understanding the function of minor collagens in cancer, we hypothesized that collagens are significantly mutated in tumors and that these mutations impact disease progression, therapy response, and patient outcomes. We further hypothesized that somatic mutations in collagens would resemble mutations observed in many collagenopathies, providing insights to function of collagens expressed and secreted from cancer cells. (15, 16). Patients with collagenopathies have both missense and truncation mutations that can be either dominant or recessive and demonstrate a range of penetrance depending on the mutation and collagen (17–20).

Collagens have not been previously reported to be significantly mutated in cancer. To our knowledge, only one formal report of recurring mutations in a collagen, COL2A1 in chondrosarcoma, has been published (21). Screening by MutSig2CV across 27 cancers identified COL11A1, COL13A1, COL19A1, COL1A2, and COL4A4 as borderline significantly mutated (22), and 2 collagens were significant in the TCGA STAD

(Table S1). Functional studies of these variants were not pursued in larger -omic screens in part due to "technical limitations", as stated by the authors (22). COL14A1 was reported to have a nonsynonymous mutation rate of 4.4% in Microsatellite Stable (MSS) gastric tumors, but no further characterization was pursued (23). Grouping genes either by network methods (24) or by careful examination of specific gene families and mutation has provided insights into splicing regulators (25), TGF-β signaling (26), and complement genes (27), for example. Because there is some redundancy and overlap in function of collagens, we applied this approach to consider the collagens as a group, and to identify sets of collagens that may be significantly mutated and impactful in stomach cancer.

In this article, we use bioinformatics to elucidate how collagen mutations affect gastric tumor outcomes. First, we find that many expressed collagens harbor somatic missense and truncation mutations, at a higher rate than expected compared to the background mutation rate. Next, we show that collagen genes and combinations of these genes associate with differential patient outcomes. We further investigate how collagen mutations correlate with tumor hallmarks, extracellular matrix components, and immune infiltration. Together, these findings suggest that collagen mutations impact stomach tumors via distinct tumor microenvironments, and that many minor collagens, in particular, have unexpected novel functions in stomach tumors.

# Results

# Collagen mutations are prevalent in STAD

We evaluated the frequency of somatic mutations in the 43 human collagen genes. We observed a clear bias in the distribution of the frequency of mutations in collagens compared to other genes ($p < 1e-16$, Wilcoxon Rank test) (Fig. 1A). Five individual collagen genes are mutated at frequencies larger than 8% (Fig. 1B). Frequently mutated genes include COL12A1, COL11A1, COL6A2, and COL7A1, representing a range of collagen families and functions. To account for the range of mutation rates in stomach tumors, we evaluated the MSS and MSIH types separately and found frequent somatic mutations in collagens in both MSIH and MSS tumors (Fig. 1C). Some collagens such as COL12A1 and COL4A1 showed high mutation rates in both MSIH and MSS tumors, while others such as COL7A1 were frequently mutated in MSIH, but not MSS tumors. Every MSIH tumor has at least one mutation in a collagen gene. In MSS tumors, COL12A1 was the most frequently mutated at 8% with only 20 tumors harboring any collagen truncation mutation.

Because collagen somatic variants are relatively rare and have not previously been identified as significantly mutated by standard algorithms, we evaluated if collagen genes were significantly mutated relative to the background mutation rate using multiple approaches. By MutSigCV2, only 2 collagen genes were significantly mutated in the TCGA gastric cancer cohort while 2 other collagens had borderline q-values (Table S1). To determine the significance of somatic mutations in collagens relative to other genes, accounting for mutation rate, we applied a modified Kolmogorov-Smirnov (KS) test (27). KS test analysis revealed that as a group, mutation rate of collagens occurred significantly above

background (Fig. 1D). Because some GC tumors are MSIH with high mutation rates compared to MSS tumors, we determined that collagens and subsets of collagen genes had significantly higher mutation rates in these more specific cohorts as well (Fig. 1D).

Collagens are mutated at similar rates in other datasets.

We examined independent datasets to assess if other GC cohorts harbor similar mutations rates of collagens. 52% of tumors have at least one somatic mutation in a collagen in the Pfizer/Hong Kong whole genome sequencing dataset in 100 cases collected in Hong Kong, including a 19% rate of truncation mutations (28), but patient survival data is not available. The Asian Cancer Research Group (ACRG) performed targeted sequencing of 251 gastric tumors including a selection of collagens including COL11A1, COL12A1, COL21A1, COL22A1, COL4A1, COL5A1, COL5A3, COL6A3, and COL6A5 (29). Recurrent variants of the collagen genes tested were reported at frequencies slightly lower than observed in TCGA (**Figure S1**). Tumors harboring at least one mutation in COL11A1, COL5A1, COL5A3, COL6A3, COL6A5, or COL4A1 were moderately associated with improved outcomes (**Figure S2B**). Major differences in the studies included that ACRG only sequenced patients of Asian ethnicity compared to TCGA including mostly Caucasians. Patients in the ACRG cohort had a longer overall survival (OS) than the TCGA patients (< 50% survival vs. >60% survival at 5 years), and the MSI cases in the ACRG cohort showed a stronger association with improved outcomes compared to the TCGA cohort (**Figure S2B**).

## Collagen Mutations Associated With Clinicopathological Characteristics

Collagen mutations classify STAD tumors independently of clinicopathological characteristics including stage and grade (**Table 1**). Age at diagnosis was associated with missense mutations and the overall mutation rate, consistent with MSIH tumors' known association with older patients (30). Previous STAD classification identifies 4 major groups: EBV, High Mutation (HM), GS, and chromosome instability (CIN). Almost every tumor in HM, characterized by high mutation rates, has at least one mutation in a collagen gene, but also, 56% of the CIN and 36% of the GS groups have collagen mutations even though the mutation rates are much lower in these groups. Neither EBV nor H. Pylori status was associated with collagen mutations, or COL7A1 mutations (**Table 1**). These TCGA defined classifications were not associated with patient outcomes.

## Collagen Mutations Associated With Patient Survival

We evaluated the association of tumors harboring a somatic nonsynonymous missense or truncation mutation in any collagen with OS by Kaplan-Meier analysis in the TCGA STAD dataset (Fig. 2A). Tumors with at least one mutation in any collagen were not associated with OS, but tumors with at least one truncation mutation in any collagen were significantly associated with longer OS (Fig. 2A). Only COL5A2, COL11A1, and COL19A1 were significantly associated with longer OS when considered as individual

genes (**Figure S1**). COL23A1 was associated with shorter survival but is mutated in only 4 cases and is not significantly expressed in STAD (**Table S2**).

To address the potential redundancy of collagen functions and since many collagens were not mutated in sufficient number of cases for survival analysis, we undertook a combinatorics approach to identify sets of tumors with mutated collagen genes associated with OS more significantly than a bootstrap defined background accounting for number of patients, mutation rate and number of genes (Figs. 2B). We evaluated all combinations of tumors with at least one mutation in 2 or 3 expressed collagen genes (**Table S3**) and tested their association with OS. The combinatorics approach identified collagen gene sets associated with OS and was sensitive to both the association with OS and the distribution of the mutations across the cohort at q ≤ 0.05 (Fig. 2). COL5A2, COL4A1, COL11A1, COL15A1, and COL16A1 were the most frequently included collagen genes in the combinations (**Figure S3A**), and each at least trended with longer OS on their own (**Figure S2A**). Truncation mutations were particularly strongly associated with OS with no combinations identified associated with shorter OS at these thresholds (Fig. 2A). Of the 104 tumors with at least one truncation mutation, 65% were in MSIH tumors (**Table 1**). We identified combinations of 2 or 3 collagen genes with truncation mutations strongly associated with OS and COL12A1 was the most collagen gene most frequently included in significant sets (Fig. 2C, **Table S3**).

## Collagen Genes Classify Msih Tumors By Overall Survival

Because the large majority of collagen mutations were in MSIH cases, and because of the differences in MSIH and MSS stomach tumors in treatment response, we evaluated each of these groups separately. For simplicity and to observe differences between MSIH and MSS differences more clearly, we removed the MSIL annotated tumors to avoid complications from these tumors with moderate mutation burden that are clinically treated as MSS tumors. Even in just MSIH cases, most truncation mutations in collagens were associated with longer survival including in COL1A1, COL5A2, COL11A1, and COL15A1 (Fig. 2D. A few combination collagens were associated with shorter survival; including most notably, COL5A3. All patients with either a COL1A1 or COL5A2 truncation in MSIH tumors were associated with longer OS (Fig. 2D). In MSS tumors, COL5A3, COL6A2, COL11A1, and COL24A1 were associated with longer OS (**Figure S3C**).

Combining the top truncation variants identified by combinatorics identified tumors strongly associated with longer OS in MSS and MSIH tumors (**Figures S3E and S4**). These observations suggest that loss of expression of many collagens, especially those involved in collagen type I expression and formation, from cancer cells was associated with increased OS in both MSS and MSIH tumors. These findings suggest that loss of function of some collagens is detrimental to patients with MSIH tumors as highlighted by COL5A3 and COL14A1. These two collagens are negative regulators of fiber size, and LOF mutations in these collagens lead to gain of function of collagen type I. COL14A1 is a Fibril Associated Collagens with Interrupted Triple helices (FACIT) collagen that regulates collagen fibrillogenesis such that

absence of COL14A1 leads to larger fibers in mice (31–33). Collagen fiber width has been associated with poor outcomes in stomach cancer (13). These observations suggest that regulation of COL1A1 and fibrillogenesis, when unchecked, leads to even worse survival in MSIH cases. Analogous to the observations in stomach tumors, mutations of collagen α3(V) chains have phenotypes distinct from collagen α1(V) and α2(V) chains in mice (34). Germline mutations in COL5A1 and COL5A2 cause Ehlers-Danlos-like phenotypes, while mutations in COL5A3 affect adiposity (34). On the other hand, LOF mutations in other regulators of fiber formation such as COL11A1 (35) were associated with longer OS (**Figure S2**).

Many collagen gene combinations' associations with OS was specific for MSIH or MSS tumors (**Table S3**, **Figure S4, Fig. 3**). Representative sets were associated with OS in either MSIH or MSS tumor, but not both (Fig. 3). In particular, even though COL5A3 and COL14A1 were mutated at similar levels, these collagens showed MSI status dependent associations with OS.

We applied the cox proportional hazards model to test the relationship of collagen mutations with other common survival associated characteristics including age and stage. Multivariate analysis showed that collagen mutations were independent predictors of OS compared to other clinicopathological characteristics including stage (**Table 2**). Neither mutation rate nor MSI status were associated with OS, despite including many collagen mutations. These findings suggest that tumors with collagen mutations specifically define a class of tumors with distinct properties and treatment responses.

## Collagen mutations impact on stomach tumors

To gain insight into how collagens could be affecting STAD tumors, we used pre-ranked Gene Set Enrichment Analysis (GSEA) of TCGA normalized RSEM scores to identify biological processes associated with tumors that harbor a collagen mutation compared to tumors without collagen mutations. We first evaluated the 50 MSigDB hallmark gene sets (36) (Fig. 4). There was high similarity of the impact of collagen mutations on expression of cancer hallmarks highlighted by the higher expression of cell cycle drivers including E2F targets and MYC gene sets (Fig. 4A, **B**). On the other hand, EMT, KRAS and myogenesis gene sets were expressed higher in wild-type compared to collagen mutant tumors (Fig. 4A, **B**). Lower expression of the EMT hallmark in tumors with collagen mutations is consistent with reduced collagen function and an altered ECM that leads to more epithelial features in these tumors (37).

To test if the ECM was different in tumors with collagen mutations, we evaluated the NABA ECM gene sets. All the NABA gene sets were expressed lower in tumors with mutations compared to wild-type tumors when considering the full TCGA STAD cohort, consistent with a disrupted ECM in the collagen mutated tumors relative to the wild-type tumors (Fig. 4B). Total collagen expression has been associated with patient outcomes in many cancers (38). Although collagens as a group were expressed lower in mutant tumors, only rarely was the expression directly associated with mutation within that collagen gene (**Table S4**). This may be because many of the collagens are expressed from both cancer and stroma cells, obscuring the relationship between collagen mutation and expression in bulk RNAseq data.

# Collagen Mutations Associated With Distinct Tumor Microenvironments

Collagens can mediate the migration and infiltration of immune cells in tumors (39). To evaluate association between collagen mutations and immune cell infiltration, we evaluated immune cell signatures (40) with pre-ranked GSEA. MSIH tumors have higher expression of most of the immune cell expression signatures compared to MSS tumors, consistent with more immune cell infiltration in MSIH tumors (**Figure S5**). Together with lower expression of the NABA ECM gene sets in MSIH tumors (**Figure S5**), these observations suggest that the MSIH and MSS tumors differ in their tumor microenvironments. Immune cell gene expression signatures were expressed higher in MSIH tumors compared to MSS tumors, consistent with a more inflammatory environment and higher tumor mutation burden in MSIH compared to MSS tumors. Because of these large differences in the tumor environments of MSS and MSIH tumors, considering all the stomach tumors together may be obfuscating impacts. We therefore evaluated the impact of collagen mutations in the whole cohort as well in MSIH and MSS tumors separately.

Combinations of collagen mutations had a more consistent impact on the expression of ECM and immune cell gene signature in MSS tumors, compared to more variable associations in MSIH tumors (Fig. 4 **and Figures S6-S10**). Figure 4 shows representative combinations and pre-ranked GSEA of all combinations listed in Table S3 are shown in Figures S6-S10. The consistent nature of the tumors with collagen mutations suggests that changes in EMT, expression in basement membranes, and many immune cells, are common features of tumors with collagen mutations in both MSS and MSIH tumors. This consistency could be because we selected for tumors with collagen mutations associated with overall survival and these tumors have similar mechanisms of impact in mediating EMT and expression of the basement membrane.

In MSS tumors, tumors with collagen mutations had consistently lower expression of all the ECM NABA and the majority of the immune cell gene sets (Fig. 4C). While in MSIH tumors, the ECM NABA and immune cell signature gene sets were split into 2 groups (Fig. 4D). Notably, tumors with COL14A1 and COL5A3 mutations were associated with higher expression of NABA gene sets in MSIH tumors and shorter OS (Fig. 4D). On the other hand, other fibril associated collagens such as COL11A1 and COL5A2 were associated with longer OS (**Figure S3**, **Table S4**). COL11A1, COL5A1, and COL5A2 promote fibril formation and loss of function mutations of these collagens have been associated with smaller collagen type I fibers (34). Mutations in COL1A1, COL11A1, COL5A1, and COL5A2 were all associated with lower expression of the EMT hallmark gene set compared to wild type in MSIH tumors, while mutations in COL14A1 and COL5A3 were associated with higher expression of EMT expression signature in MSIH tumors (Fig. 4D). These observations predict that tumors with mutations in collagen types XIV and Vα3 would have thicker fibers, promoting cell migration, and subsequent tumor cell escape. On the other hand, tumors with in COL1A1, COL11A1, COL5A1, and COL5A2 are predicted to have thinner or fewer collagen

type I fibers leading to less migration, lower mesenchymal properties, less metastasis, and higher sensitivity to treatments.

# Immunoenvironment

We used the immune cell gene sets reported by Tamborero et al. to evaluate the immunoenvironment by pre-rank GSEA (40). Across the whole cohort, B and mast cells were lower in tumors with a variety of mutant collagens, while T helper cells were modestly increased in some mutant collagen tumors (Fig. 4A). In MSS tumors, Mast cells, macrophages, neutrophils were lower in mutant tumors associated with longer survival, and higher in wildtype tumors (Fig. 4B). Mast cells and neutrophils have been associated with short OS in STAD (41, 42). When considering just the MSIH tumors, neutrophils and mast cells were lower in mutant tumors, but no other clear patterns emerged across the cohort. Immunosuppressive cell types including macrophages and regulatory T cells are higher in many of the shorter OS mutation combinations in both MSS and MSIH tumors. Tumors with COL14A1 mutations for example had higher levels of macrophages, neutrophils and mast cells Mast cells have been associated with shorter OS in stomach cancer (41). These observations, based on molecular signatures, suggest changes to the immunoenvironment in tumors with collagen mutations.

COL7A1 mutations

We hypothesized that insight into the functional impact of mutations can be gained by comparing the pattern and type of mutation to those observed in collagenopathies. As an example, we focused on COL7A1 which is the mutational cause of Dystrophic Epidermolysis Bullosa (DEB) and had significant associations with patient outcomes in MSIH tumors (Fig. 2B). COL7A1 germline mutations were downloaded from a DEB mutation database (43). The distribution of germline and stomach somatic mutations were very similar (**Figure S11B**). A Kruskal-Wallis test ($P$ = 0.3) suggested that the two distributions were not significantly different. COL7A1 mutations in STAD were slightly more biased towards the N-terminus of the protein compared to DEB. The largest exon, exon 73, was most frequently mutated in both the germline and cancer mutations. The recurring nonsense mutation at position 2029 is the same hotspot observed in DEB patients (**Figure S11A and B**). The distribution of somatic mutations resembled the distribution of DEB germline mutations suggesting that no unusual tumor specific mutation pattern is prevalent in stomach tumors. Moreover, because the type of mutation is similar as observed in DEB, we can infer the function of the somatic mutations in tumors.

Expression of COL7A1 in stomach tumors.

Many minor collagens have a high tissue specificity including COL7A1 (44). Because COL7A1 is not known to be expressed in normal stomach or anywhere in the gastrointestinal tract, we wanted to confirm that COL7A1 was actually expressed in stomach tumors, and importantly determine if COL7A1 was expressed in cancer cells and not just in the stroma; a necessary requirement for COL7A1 mutations to be meaningful. We evaluated COL7A1 protein expression by immunohistochemistry in a set of 10 stomach

tumors from patients treated at Rhode Island Hospital (**Figure S12**). COL7A1 expression was expressed in the stroma (4/10), in the epithelium (3/10) or was not detectable (3/10) (**Table S5**). In the epithelium, COL7A1 was expressed in the cytoplasm. Expression from tumor cells was confirmed by *in situ* hybridization using RNAscope (**Figure S12A**). Larger studies are needed to evaluate any connection between COL7A1 expression patterns and mutation status.

## Discussion

This work demonstrates that collagen deposition by cancer cells is affected by a significant mutation rate, which is associated with myriad tumor features and patient outcomes. Other collagens have been shown to be expressed in tumor cells by *in situ* hybridization such as collagen type IV (9). Hynes and colleagues have suggested that matrisome components secreted from tumors cells in pancreatic tumors are more impactful than those originating from the stroma (45). It is likely that cancer cells locally deposit collagen, which could disproportionately affect other nearby cancer cells, relative to collagen secreted by stromal or immune cells elsewhere in the tumor. Unexpectedly, truncation mutations in collagens had strong association with longer OS suggesting that a reduction of collagen expression in cancer cells is beneficial to patients for many collagens. Truncation mutations were the most strongly associated with outcomes. This is likely because truncation mutations are more uniform in their impact than missense variants, which include both loss and gain of function phenotypes. Interpreting collagen mutations as originating from cancer cells provides a new approach to interpret tumor cell secreted matrisome factors. Insights from mutations could be applied to other ECM factors originating from both tumor and stroma cells. Consistent with these reports, the observations reported here suggest that collagens secreted from cancer cells are critical for tumor progress and response to treatment. These data strongly support the concept that the collagens secreted from cancer cells are vital to shape the tumor environment.

We made discoveries on the role of myriad collagens in stomach cancer. COL12A1 was the collagen gene with the highest truncation mutation frequency and most often strongly associated with OS in the combinatorics analysis (Fig. 2). COL12A1 is a FACIT homotrimer collagen that mediates interactions between collagen type I and the rest of the ECM. COL12A1 is reported to be expressed by both stroma and tumor cells in STAD (46–49) and is expressed in gastric cancer cell lines (50). Together, these observations suggest that COL12A1, originating from cancer cells, is a critical determinant of STAD disease progression and therapy response.

The dysregulated expression of COL7A1 has not previously been reported in solid tumors. COL7A1 RNA expression was not associated with outcome in TCGA (data not shown). This is likely because COL7A1 expression from either tumor or stroma cells could have distinct impacts on therapy response and patient outcomes, as proposed by recent findings comparing secreted proteins from cancer and stroma cells (45). Analysis of mutations distinguishes impacts of COL7A1 in tumors cells from those in stroma cells that is not discernable from RNA expression data alone.

Collagens form two of the major structures in the ECM: the basement membrane and the collagen type I fiber network. Both of these ECM components are impacted by collagen mutations. The loss of integrity of the basement membrane in tumors suggests a disorganized, more porous structure that could cause increased inflammation, analogous to COL7A1 mutations in DEB, or with collagen type IV and type VI variants (16). Collagen type I, expressed by the COL1A1 and COL1A2 genes, plays a critical role in forming the ECM and organizing cell-cell interactions and mechanical properties in tumors (51). Increased collagen type I has been associated with worse outcomes in many cancers including stomach (13, 52). Both COL1A1 and COL1A2 had modest mutation frequencies with only weak association with OS in STAD (Fig. 1; **Figure S2**). However, truncation mutations of COL1A1 were associated with longer survival and most truncation mutations of COL1A2 were also associated with longer survival except for 1 case, TCGA-HU-A4GQ-01, which was reported to have deceased at 0 months, and therefore may be reflecting other causes of death. Collagen type I missense variants were not associated with OS, perhaps because the majority of collagen type I originates from the stroma and therefore any impact of a mutated collagen type I originating from tumor cells may be diluted. Collagens that interact with collagen type I and regulate fiber size and structure including all 3 collagen type V genes, COL11A1, COL12A1, and COL14A1 have significant mutation rates and association with OS. These observations further support the concept that loss of collagen type I from the tumor cells, or dysregulation of the network that forms collagen type I dependent structures affects patient outcomes.

Altogether, these observations suggest the regulation of collagen type I and the basement membrane by a panoply of cancer cell secreted collagens are critical for tumor fate. Collagens, as one of the dominant structural proteins in the ECM play myriad functions in regulating cancer hallmarks (53). Minor collagens such as COL7A1 and COL12A1 form structural links between the collagen type I fiber network and/or the basement membrane zone. These data support a model where a local ECM derived from components secreted from the cancer cells, reshape the local ECM and are critical for tumor phenotypes, including EMT, drug response, the immunoenvironment and overall disease progression (Fig. 5). In tumors with wild-type collagens, EMT is higher, collagen type I fibers are wider, and higher expression of the matrisome including the basement membrane compared to tumors with mutant collagens. On the other hand, some mutant MSIH tumors, associated with shorter OS, exemplified by missense COL5A3 and COL14A1 which regulate COL1A1, have higher expression of mesenchymal genes, wider collagen type I fibers, and a different immune cell infiltration pattern (Fig. 4D). Linking hallmarks and pathways to dysregulated ECM caused by collagen mutations may lead to new opportunities to refine drug targeting and development.

In conclusion, we find a high frequency of individual collagen genes and sets of collagen genes harboring somatic mutations compared to background in both microsatellite stable (MSS), and microsatellite instable (MSIH) stomach adenocarcinomas in TCGA and comparable datasets. Overall, combinations of somatic mutations are predictive of patient survival, and truncation mutations associate with improved survival. We further associate these combinations with distinctive tumor microenvironments based on lower matrisome expression, cell cycle and EMT, as well as immune cell infiltration. Interestingly, stomach cells express COL7A1, normally associated with skin ECM, and somatic mutations in COL7A1 predict

improved survival. It should be noted that this study is limited by the dependence on genotype-phenotype correlations in patients so that there is risk in oversimplifying rare mutations. Some of this risk is ameliorated by the combinatorial approach and the interpretation of the mutations based on similar variants observed in collagenopathies. Nevertheless, many of these missense mutations resemble the loss of function (LOF) mutations in collagenopathies, which may give some insight into their role in tumor progression. Overall, this study suggests the further testing of collagen mutations in stomach cancer is promising and collagen mutations could be incorporated into strategies to classify GC patients. Such patterns of collagen mutations may be more widely applicable beyond stomach cancers to other solid tumor types.

# Methods

Data sources

The Cancer Genome Atlas (TCGA) Pan-Cancer RNA-seq V2 normalized gene expression and clinical data was downloaded from Firebrowse in April 2018. TCGA somatic mutation data file, mc3.v0.2.8.PUBLIC.maf, was downloaded from the Genomic Data Commons (GDC) (55). Microsatellite data was downloaded from Firebrowse (STAD.merged_only_auxillary_clin_format.txt). Immune gene sets input into GSEA were defined by Tamborero et al. (40). Stroma scores, and overall mutation rates were derived from Table S1 by Thorsson et al (56). Hallmark (57)and NABA (58)gene sets were downloaded from MsigDB v7.0 (36). For the analysis of collagen mutations in the ACRG, collagen mutation data was obtained from the supplemental data published by Cristescu et al. (29).

Software and statistical tests.

Analyses were performed using R and python custom scripts. GSEA version 2.4 was run on either a Unix or MacOS system. Statistical tests were performed using the Lifelines v0.25.1 and SciPy v1.5.2

libraries in Python. Moderated Kolmogorov-Smirnov test was adopted from Olcina et al. to assess significance of collagen somatic mutations relative to other genes (27). Morpheus was used to generate the heatmaps (54). Survival curves were generated using cBioPortal's oncoprinter web app and matplotlib v3.3.1.

Identifying collagen gene combinations.

We aimed to identify sets of collagen genes significantly associated with overall survival, accounting for gene size and mutation rate. To correct for multiple combinations occurring by chance, we calculated a q value for a given subset of collagen genes. To determine background, genes were randomly chosen until the expected number of mutations were within 5 of the number of observed mutations in collagen genes. A survival analysis was performed on the subset of patients used in the collagen subset analysis where the indicator variable was based on whether a patient has a mutation in the randomly chosen subset in at least 5% of cases of the designated cohort. We considered subsets with collagen genes significantly

expressed with an average RSEM > 200. If a combination of 2 collagens was identified, this combination was not considered in combinations of 3 collagens. We then counted the frequency of each collagen included in the subsets as an indication of the contribution of each collagen to overall survival risk and exclusivity with the other collagens.

## Case selection for immunohistochemistry

With institutional review board approval, IRB #1070389-9, 10 cases of gastric adenocarcinoma diagnosed from 2010 to 2019 were retrieved from the archives of the Department of Pathology and Laboratory Medicine at Lifespan Academic Medical Center (Providence, RI).

## Immunohistochemistry

Immunohistochemistry staining for COL7A1 was performed on 4-µm paraffin sections. After incubation at 60 °C for 30 minutes, the sections were deparaffinized and rehydrated with xylene and graded alcohols. Antigen retrieval was performed with Ready-to-Use Proteinase K (Agilent, Santa Clara, CA) incubating at 37 °C for 10 minutes. The slides were then incubated with anti-COL7A1 antibody (1:5000) for overnight at 4 °C. The immunoreactivity was detected by using the DAKO Envision + Dual Link System and the DAKO Liquid 3,3'-diaminobenzidine (DAB+) Substrate Chromagen System (Agilent, Santa Clara, CA). Immunohistochemistry was assessed by 2 pathologists (MR and EW).

## In situ hybridization

mRNA expression was determined using ISH with the RNAscope Assay (Advanced Cell Diagnostics, Hayward, CA). The ISH staining for COL7A1 was performed on 4-µm paraffin sections. After baking slides at 60 °C for 1 hour and deparaffinizing FFPE sections with xylene, RNAscope® 2.5 HD Reagent Kit was used for the ISH assay. All the steps were done according to the kit protocol. After pre-treating the sample with hydrogen peroxide solution, heat target retrieval and protease plus, COL7A1 probe was added for 2hr at 40 °C, sequentially hybridize with AMP 1, AMP 2, AMP 3, AMP 4, AMP 5, and AMP 6 reagents, for 30, 15, 30, 15, 60, 15 min, respectively. ISH signal was detected by the application of a chromogenic substrate. Tissue was counter-stained with haematoxylin. Scrambled negative control probes showed no signal.

## Antibody sources

Rabbit polyclonal anti-COL7A1 targeting the human LH7.2 domain was a kind gift from Alexander Nystrom, University of Freiburg (59).

# Declarations

## *Availability of Data and Materials*

All genomic data used in this study is publicly available.

# References

1. Egeblad M, Nakasone ES, Werb Z. Tumors as organs: complex tissues that interface with the entire organism. Dev Cell. 2010;18(6):884–901. doi:10.1016/j.devcel.2010.05.012. PubMed PMID: 20627072; PMCID: PMC2905377. Epub 2010/07/16.

2. Nissen NI, Karsdal M, Willumsen N. Collagens and Cancer associated fibroblasts in the reactive stroma and its relation to Cancer biology. J Exp Clin Cancer Res. 2019;38(1):115. doi:10.1186/s13046-019-1110-6. PubMed PMID: 30841909; PMCID: PMC6404286. Epub 2019/03/08.

3. Becht E, de Reynies A, Giraldo NA, Pilati C, Buttard B, Lacroix L, Selves J, Sautes-Fridman C, Laurent-Puig P, Fridman WH. Immune and Stromal Classification of Colorectal Cancer Is Associated with Molecular Subtypes and Relevant for Precision Immunotherapy. Clin Cancer Res. 2016;22(16):4057–66. doi:10.1158/1078-0432.CCR-15-2879. PubMed PMID: 26994146.

4. Fang M, Yuan J, Peng C, Li Y. Collagen as a double-edged sword in tumor progression. Tumour Biol. 2014;35(4):2871–82. doi:10.1007/s13277-013-1511-7. PubMed PMID: 24338768; PMCID: PMC3980040.

5. Ricard-Blum S. The collagen family. Cold Spring Harb Perspect Biol. 2011;3(1):a004978. doi:10.1101/cshperspect.a004978. PubMed PMID: 21421911; PMCID: PMC3003457.

6. Brodsky AS, Xiong J, Yang D, Schorl C, Fenton MA, Graves TA, Sikov WM, Resnick MB, Wang Y. Identification of stromal ColXalpha1 and tumor-infiltrating lymphocytes as putative predictive markers of neoadjuvant therapy in estrogen receptor-positive/HER2-positive breast cancer. BMC Cancer. 2016;16(1):274. doi:10.1186/s12885-016-2302-5. PubMed PMID: 27090210; PMCID: PMC4835834.

7. Jia D, Liu Z, Deng N, Tan TZ, Huang RY, Taylor-Harding B, Cheon DJ, Lawrenson K, Wiedemeyer WR, Walts AE, Karlan BY, Orsulic S. A COL11A1-correlated pan-cancer gene signature of activated fibroblasts for the prioritization of therapeutic targets. Cancer Lett. 2016;382(2):203–14. doi:10.1016/j.canlet.2016.09.001. PubMed PMID: 27609069.

8. Soini Y, Hurskainen T, Hoyhtya M, Oikarinen A, Autio-Harmainen H. 72 KD and 92 KD type IV collagenase, type IV collagen, and laminin mRNAs in breast cancer: a study by in situ hybridization. J Histochem Cytochem. 1994;42(7):945–51. doi:10.1177/42.7.8014478. PubMed PMID: 8014478. Epub 1994/07/01.

9. LI N, Sun H, Wang X, Zhang Z, Zhou Y, Anderson C, Ma X-J, editors. Extracellular matrix gene expression and cytotoxic T lymphocyte infiltration in the tumor microenvironment in non-small cell lung cancer. AACR Annual Meeting 2019; 2019 Mar 29-Apr 3 2019; Atlanta, GA: AACR; 2019.

10. Tian C, Clauser KR, Ohlund D, Rickelt S, Huang Y, Gupta M, Mani DR, Carr SA, Tuveson DA, Hynes RO. Proteomic analyses of ECM during pancreatic ductal adenocarcinoma progression reveal different contributions by tumor and stromal cells. Proc Natl Acad Sci U S A. 2019. doi:10.1073/pnas.1908626116. PubMed PMID: 31484774. Epub 2019/09/06.

11. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Pineros M, Znaor A, Bray F. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer. 2019;144(8):1941–53. doi:10.1002/ijc.31937. PubMed PMID: 30350310. Epub 2018/10/24.

12. Song Z, Wu Y, Yang J, Yang D, Fang X. Progress in the treatment of advanced gastric cancer. Tumour Biol. 2017;39(7):1010428317714626. i: 10.1177/1010428317714626. PubMed PMID: 28671042.. ; ). do.

13. Zhou ZH, Ji CD, Xiao HL, Zhao HB, Cui YH, Bian XW. Reorganized Collagen in the Tumor Microenvironment of Gastric Cancer and Its Association with Prognosis. J Cancer. 2017;8(8):1466–76. doi:10.7150/jca.18466. PubMed PMID: 28638462; PMCID: PMC5479253.

14. Chen D, Chen G, Jiang W, Fu M, Liu W, Sui J, Xu S, Liu Z, Zheng X, Chi L, Lin D, Li K, Chen W, Zuo N, Lu J, Chen J, Li G, Zhuo S, Yan J. Association of the Collagen Signature in the Tumor Microenvironment With Lymph Node Metastasis in Early Gastric Cancer. JAMA Surg. 2019:e185249. Epub 2019/01/31. doi:10.1001/jamasurg.2018.5249. PubMed PMID: 30698615.

15. Zankl A, Neumann L, Ignatius J, Nikkels P, Schrander-Stumpel C, Mortier G, Omran H, Wright M, Hilbert K, Bonafe L, Spranger J, Zabel B, Superti-Furga A. Dominant negative mutations in the C-propeptide of COL2A1 cause platyspondylic lethal skeletal dysplasia, torrance type, and define a novel subfamily within the type 2 collagenopathies. Am J Med Genet A. 2005;133A(1):61–7. doi:10.1002/ajmg.a.30531. PubMed PMID: 15643621.

16. Jobling R, D'Souza R, Baker N, Lara-Corrales I, Mendoza-Londono R, Dupuis L, Savarirayan R, Ala-Kokko L, Kannu P. The collagenopathies: review of clinical phenotypes and molecular correlations. Curr Rheumatol Rep. 2014;16(1):394. doi:10.1007/s11926-013-0394-3. PubMed PMID: 24338780.

17. Christiano AM, Ryynanen M, Uitto J. Dominant dystrophic epidermolysis bullosa: identification of a Gly−>Ser substitution in the triple-helical domain of type VII collagen. Proc Natl Acad Sci U S A. 1994;91(9):3549−53. PubMed PMID: 8170945; PMCID: PMC43617.

18. Kuivaniemi H, Tromp G, Prockop DJ. Mutations in collagen genes: causes of rare and some common diseases in humans. FASEB J. 1991;5(7):2052−60. PubMed PMID: 2010058.

19. Spranger J, Winterpacht A, Zabel B. The type II collagenopathies: a spectrum of chondrodysplasias. Eur J Pediatr. 1994;153(2):56−65. PubMed PMID: 8157027.

20. Vikkula M, Metsaranta M, Ala-Kokko L. Type II collagen mutations in rare and common cartilage diseases. Ann Med. 1994;26(2):107−14. PubMed PMID: 8024727.

21. Tarpey PS, Behjati S, Cooke SL, Van Loo P, Wedge DC, Pillay N, Marshall J, O'Meara S, Davies H, Nik-Zainal S, Beare D, Butler A, Gamble J, Hardy C, Hinton J, Jia MM, Jayakumar A, Jones D, Latimer C, Maddison M, Martin S, McLaren S, Menzies A, Mudie L, Raine K, Teague JW, Tubio JM, Halai D, Tirabosco R, Amary F, Campbell PJ, Stratton MR, Flanagan AM, Futreal PA. Frequent mutation of the major cartilage collagen gene COL2A1 in chondrosarcoma. Nat Genet. 2013;45(8):923−6. doi:10.1038/ng.2668. PubMed PMID: 23770606; PMCID: PMC3743157.

22. Kim E, Ilic N, Shrestha Y, Zou L, Kamburov A, Zhu C, Yang X, Lubonja R, Tran N, Nguyen C, Lawrence MS, Piccioni F, Bagul M, Doench JG, Chouinard CR, Wu X, Hogstrom L, Natoli T, Tamayo P, Horn H, Corsello SM, Lage K, Root DE, Subramanian A, Golub TR, Getz G, Boehm JS, Hahn WC. Systematic Functional Interrogation of Rare Cancer Variants Identifies Oncogenic Alleles. Cancer Discov. 2016;6(7):714−26. doi:10.1158/2159-8290.CD-16-0160. PubMed PMID: 27147599; PMCID: PMC4930723.

23. Li X, Wu WK, Xing R, Wong SH, Liu Y, Fang X, Zhang Y, Wang M, Wang J, Li L, Zhou Y, Tang S, Peng S, Qiu K, Chen L, Chen K, Yang H, Zhang W, Chan MT, Lu Y, Sung JJ, Yu J. Distinct Subtypes of Gastric Cancer Defined by Molecular Characterization Include Novel Mutational Signatures with Prognostic Capability. Cancer Res. 2016;76(7):1724−32. doi:10.1158/0008-5472.CAN-15-2443. PubMed PMID: 26857262.

24. Leiserson MD, Vandin F, Wu HT, Dobson JR, Eldridge JV, Thomas JL, Papoutsaki A, Kim Y, Niu B, McLellan M, Lawrence MS, Gonzalez-Perez A, Tamborero D, Cheng Y, Ryslik GA, Lopez-Bigas N, Getz G, Ding L, Raphael BJ. Pan-cancer network analysis identifies combinations of rare somatic mutations across pathways and protein complexes. Nat Genet. 2015;47(2):106−14. doi:10.1038/ng.3168. PubMed PMID: 25501392; PMCID: 4444046.

25. Zhou B, Wang GZ, Wen ZS, Zhou YC, Huang YC, Chen Y, Zhou GB. Somatic Mutations and Splicing Variants of Focal Adhesion Kinase in Non-Small Cell Lung Cancer. J Natl Cancer Inst. 2018;110(2). doi:10.1093/jnci/djx157. PubMed PMID: 29087503.

26. Korkut A, Zaidi S, Kanchi RS, Rao S, Gough NR, Schultz A, Li X, Lorenzi PL, Berger AC, Robertson G, Kwong LN, Datto M, Roszik J, Ling S, Ravikumar V, Manyam G, Rao A, Shelley S, Liu Y, Ju Z, Hansel D, de Velasco G, Pennathur A, Andersen JB, O'Rourke CJ, Ohshiro K, Jogunoori W, Nguyen BN, Li S, Osmanbeyoglu HU, Ajani JA, Mani SA, Houseman A, Wiznerowicz M, Chen J, Gu S, Ma W, Zhang J, Tong P, Cherniack AD, Deng C, Resar L, Cancer Genome Atlas Research N, Weinstein JN, Mishra L, Akbani R. A Pan-Cancer Analysis Reveals High-Frequency Genetic Alterations in Mediators of Signaling by the TGF-beta Superfamily. Cell Syst. 2018;7(4):422 – 37 e7. Epub 2018/10/01. doi: 10.1016/j.cels.2018.08.010. PubMed PMID: 30268436.

27. Olcina MM, Balanis NG, Kim RK, Aksoy BA, Kodysh J, Thompson MJ, Hammerbacher J, Graeber TG, Giaccia AJ. Mutations in an Innate Immunity Pathway Are Associated with Poor Overall Survival Outcomes and Hypoxic Signaling in Cancer. Cell Rep. 2018;25(13):3721–32. doi: 10.1016/j.celrep.2018.11.093. PubMed PMID: 30590044.

28. Wang K, Yuen ST, Xu J, Lee SP, Yan HH, Shi ST, Siu HC, Deng S, Chu KM, Law S, Chan KH, Chan AS, Tsui WY, Ho SL, Chan AK, Man JL, Foglizzo V, Ng MK, Chan AS, Ching YP, Cheng GH, Xie T, Fernandez J, Li VS, Clevers H, Rejto PA, Mao M, Leung SY. Whole-genome sequencing and comprehensive molecular profiling identify new driver mutations in gastric cancer. Nat Genet. 2014;46(6):573–82. doi:10.1038/ng.2983. PubMed PMID: 24816253.

29. Cristescu R, Lee J, Nebozhyn M, Kim KM, Ting JC, Wong SS, Liu J, Yue YG, Wang J, Yu K, Ye XS, Do IG, Liu S, Gong L, Fu J, Jin JG, Choi MG, Sohn TS, Lee JH, Bae JM, Kim ST, Park SH, Sohn I, Jung SH, Tan P, Chen R, Hardwick J, Kang WK, Ayers M, Hongyue D, Reinhard C, Loboda A, Kim S, Aggarwal A. Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes. Nat Med. 2015;21(5):449–56. doi:10.1038/nm.3850. PubMed PMID: 25894828.

30. Ratti M, Lampis A, Hahne JC, Passalacqua R, Valeri N. Microsatellite instability in gastric cancer: molecular bases, clinical perspectives, and new treatment approaches. Cell Mol Life Sci. 2018;75(22):4151–62. doi:10.1007/s00018-018-2906-9. PubMed PMID: 30173350; PMCID: PMC6182336. Epub 2018/09/03.

31. Ansorge HL, Meng X, Zhang G, Veit G, Sun M, Klement JF, Beason DP, Soslowsky LJ, Koch M, Birk DE. Type XIV Collagen Regulates Fibrillogenesis: Premature Collagen Fibril Growth and Tissue Dysfunction in Null Mice. J Biol Chem. 2009;284(13):8427–38. doi:10.1074/jbc.M805582200. PubMed PMID: 19136672; PMCID: PMC2659201. Epub 2009/01/13.

32. Young BB, Zhang G, Koch M, Birk DE. The roles of types XII and XIV collagen in fibrillogenesis and matrix assembly in the developing cornea. J Cell Biochem. 2002;87(2):208–20. doi:10.1002/jcb.10290. PubMed PMID: 12244573. Epub 2002/09/24.

33. Tao G, Levay AK, Peacock JD, Huk DJ, Both SN, Purcell NH, Pinto JR, Galantowicz ML, Koch M, Lucchesi PA, Birk DE, Lincoln J. Collagen XIV is important for growth and structural integrity of the myocardium. J Mol Cell Cardiol. 2012;53(5):626–38. doi: 10.1016/j.yjmcc.2012.08.002. PubMed PMID: 22906538; PMCID: PMC3472103.

34. Mak KM, Png CY, Lee DJ. Type V Collagen in Health, Disease, and Fibrosis. Anat Rec (Hoboken). 2016;299(5):613–29. doi: 10.1002/ar.23330. PubMed PMID: 26910848.

35. Grassel S, Bauer RJ. Collagen XVI in health and disease. Matrix Biol. 2013;32(2):64–73. doi:10.1016/j.matbio.2012.11.001. PubMed PMID: 23149016. Epub 2012/11/15.

36. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A. 2005;102(43):15545–50. doi:10.1073/pnas.0506580102. PubMed PMID: 16199517; PMCID: 1239896.

37. Jung HY, Fattet L, Yang J. Molecular pathways: linking tumor microenvironment to epithelial-mesenchymal transition in metastasis. Clin Cancer Res. 2015;21(5):962–8. doi:10.1158/1078-0432.CCR-13-3173. PubMed PMID: 25107915; PMCID: PMC4320988. Epub 2014/08/12.

38. Martins Cavaco AC, Damaso S, Casimiro S, Costa L. Collagen biology making inroads into prognosis and treatment of cancer progression and metastasis. Cancer Metastasis Rev. 2020;39(3):603–23. doi:10.1007/s10555-020-09888-5. PubMed PMID: 32447477. Epub 2020/05/25.

39. Bonnans C, Chou J, Werb Z. Remodelling the extracellular matrix in development and disease. Nat Rev Mol Cell Biol. 2014;15(12):786–801. doi:10.1038/nrm3904.

40. Tamborero D, Rubio-Perez C, Muinos F, Sabarinathan R, Piulats JM, Muntasell A, Dienstmann R, Lopez-Bigas N, Gonzalez-Perez A. A Pan-cancer Landscape of Interactions between Solid Tumors and Infiltrating Immune Cell Populations. Clin Cancer Res. 2018;24(15):3717–28. doi:10.1158/1078-0432.CCR-17-3509. PubMed PMID: 29666300. Epub 2018/04/19.

41. Lv YP, Peng LS, Wang QH, Chen N, Teng YS, Wang TT, Mao FY, Zhang JY, Cheng P, Liu YG, Kong H, Wu XL, Hao CJ, Chen W, Zhu J, Han B, Ma Q, Li K, Zou Q, Zhuang Y. Degranulation of mast cells induced by gastric cancer-derived adrenomedullin prompts gastric cancer progression. Cell Death Dis. 2018;9(10):1034. Epub 2018/10/12. doi: 10.1038/s41419-018-1100-1. PubMed PMID: 30305610; PMCID: PMC6180028.

42. Hiramatsu S, Tanaka H, Nishimura J, Sakimura C, Tamura T, Toyokawa T, Muguruma K, Yashiro M, Hirakawa K, Ohira M. Neutrophils in primary gastric tumors are correlated with neutrophil infiltration in tumor-draining lymph nodes and the systemic inflammatory response. BMC Immunol. 2018;19(1):13. doi:10.1186/s12865-018-0251-2. PubMed PMID: 29661142; PMCID: PMC5902874. Epub 2018/04/18.

43. Wertheim-Tysarowska K, Sobczynska-Tomaszewska A, Kowalewski C, Skronski M, Swieckowski G, Kutkowska-Kazmierczak A, Wozniak K, Bal J. The COL7A1 mutation database. Hum Mutat. 2012;33(2):327–31. doi:10.1002/humu.21651. PubMed PMID: 22058051. Epub 2011/11/08.

44. Bornert O, Nystrom A Cloning and Mutagenesis Strategies for Large Collagens. Methods Mol Biol. 2019;1944:3–15. Epub 2019/03/07. doi: 10.1007/978-1-4939-9095-5_1. PubMed PMID: 30840231.

45. Tian C, Ohlund D, Rickelt S, Lidstrom T, Huang Y, Hao L, Zhao RT, Franklin O, Bhatia SN, Tuveson DA, Hynes RO. Cancer-cell-derived matrisome proteins promote metastasis in pancreatic ductal adenocarcinoma. Cancer Res. 2020. Epub 2020/02/08. doi:10.1158/0008-5472.CAN-19-2578. PubMed PMID: 32029550.

46. Duan S, Gong B, Wang P, Huang H, Luo L, Liu F. Novel prognostic biomarkers of gastric cancer based on gene expression microarray: COL12A1, GSTA3, FGA and FGG. Mol Med Rep. 2018;18(4):3727−36. doi:10.3892/mmr.2018.9368. PubMed PMID: 30106150; PMCID: PMC6131538. Epub 2018/08/15.

47. Jiang X, Wu M, Xu X, Zhang L, Huang Y, Xu Z, He K, Wang H, Wang H, Teng L. COL12A1, a novel potential prognostic factor and therapeutic target in gastric cancer. Mol Med Rep. 2019. Epub 2019/08/23. doi:10.3892/mmr.2019.10548. PubMed PMID: 31432110.

48. Uhlen M, Zhang C, Lee S, Sjostedt E, Fagerberg L, Bidkhori G, Benfeitas R, Arif M, Liu Z, Edfors F, Sanli K, von Feilitzen K, Oksvold P, Lundberg E, Hober S, Nilsson P, Mattsson J, Schwenk JM, Brunnstrom H, Glimelius B, Sjoblom T, Edqvist PH, Djureinovic D, Micke P, Lindskog C, Mardinoglu A, Ponten F. A pathology atlas of the human cancer transcriptome. Science. 2017;357(6352). Epub 2017/08/19. doi:10.1126/science.aan2507. PubMed PMID: 28818916.

49. Human. Protein Atlas available from http://www.proteinatlas.org.

50. Xiang Z, Li J, Song S, Wang J, Cai W, Hu W, Ji J, Zhu Z, Zang L, Yan R, Yu Y. A positive feedback between IDO1 metabolite and COL12A1 via MAPK pathway to promote gastric cancer metastasis. J Exp Clin Cancer Res. 2019;38(1):314. doi:10.1186/s13046-019-1318-5. PubMed PMID: 31315643; PMCID: PMC6637527. Epub 2019/07/19.

51. Xu S, Xu H, Wang W, Li S, Li H, Li T, Zhang W, Yu X, Liu L. The role of collagen in cancer: from bench to bedside. J Transl Med. 2019;17(1):309. doi:10.1186/s12967-019-2058-1. PubMed PMID: 31521169. Epub 2019/09/16.

52. Ohno S, Tachibana M, Fujii T, Ueda S, Kubota H, Nagasue N. Role of stromal collagen in immunomodulation and prognosis of advanced gastric carcinoma. Int J Cancer. 2002;97(6):770−4. PubMed PMID: 11857352.

53. Pickup MW, Mouw JK, Weaver VM. The extracellular matrix modulates the hallmarks of cancer. EMBO Rep. 2014;15(12):1243−53. doi:10.15252/embr.201439246. PubMed PMID: 25381661.

54. Morpheus. Available from: https://software.broadinstitute.org/morpheus.

55. Ellrott K, Bailey MH, Saksena G, Covington KR, Kandoth C, Stewart C, Hess J, Ma S, Chiotti KE, McLellan M, Sofia HJ, Hutter C, Getz G, Wheeler D, Ding L, Group MCW. Cancer Genome Atlas Research N. Scalable Open Science Approach for Mutation Calling of Tumor Exomes Using Multiple Genomic Pipelines. Cell Syst. 2018;6(3):271−81. doi: 10.1016/j.cels.2018.03.002. PubMed PMID: 29596782; PMCID: PMC6075717.

56. Thorsson V, Gibbs DL, Brown SD, Wolf D, Bortone DS, Ou Yang TH, Porta-Pardo E, Gao GF, Plaisier CL, Eddy JA, Ziv E, Culhane AC, Paull EO, Sivakumar IKA, Gentles AJ, Malhotra R, Farshidfar F, Colaprico A, Parker JS, Mose LE, Vo NS, Liu J, Liu Y, Rader J, Dhankani V, Reynolds SM, Bowlby R, Califano A, Cherniack AD, Anastassiou D, Bedognetti D, Rao A, Chen K, Krasnitz A, Hu H, Malta TM, Noushmehr H, Pedamallu CS, Bullman S, Ojesina AI, Lamb A, Zhou W, Shen H, Choueiri TK, Weinstein JN, Guinney J, Saltz J, Holt RA, Rabkin CE, Cancer Genome Atlas Research N, Lazar AJ, Serody JS, Demicco EG, Disis ML, Vincent BG, Shmulevich L. The Immune Landscape of Cancer. Immunity. 2018;48(4):812 −

30 e14. Epub 2018/04/10. doi: 10.1016/j.immuni.2018.03.023. PubMed PMID: 29628290; PMCID: PMC5982584.

57. Liberzon A, Birger C, Thorvaldsdottir H, Ghandi M, Mesirov JP, Tamayo P. The Molecular Signatures Database (MSigDB) hallmark gene set collection. Cell Syst. 2015;1(6):417–25. doi: 10.1016/j.cels.2015.12.004. PubMed PMID: 26771021; PMCID: PMC4707969.

58. Naba A, Clauser KR, Hoersch S, Liu H, Carr SA, Hynes RO. The matrisome: in silico definition and in vivo characterization by proteomics of normal and tumor extracellular matrices. Mol Cell Proteomics. 2012;11(4):M111 014647. Epub 2011/12/14. doi: 10.1074/mcp.M111.014647. PubMed PMID: 22159717; PMCID: PMC3322572.

59. Kuhl T, Mezger M, Hausser I, Handgretinger R, Bruckner-Tuderman L, Nystrom A. High Local Concentrations of Intradermal MSCs Restore Skin Integrity and Facilitate Wound Healing in Dystrophic Epidermolysis Bullosa. Mol Ther. 2015;23(8):1368–79. doi: 10.1038/mt.2015.58. PubMed PMID: 25858020; PMCID: PMC4817872.

# Tables

Due to technical limitations, table 1 and 2 is only available as a download in the Supplemental Files section.
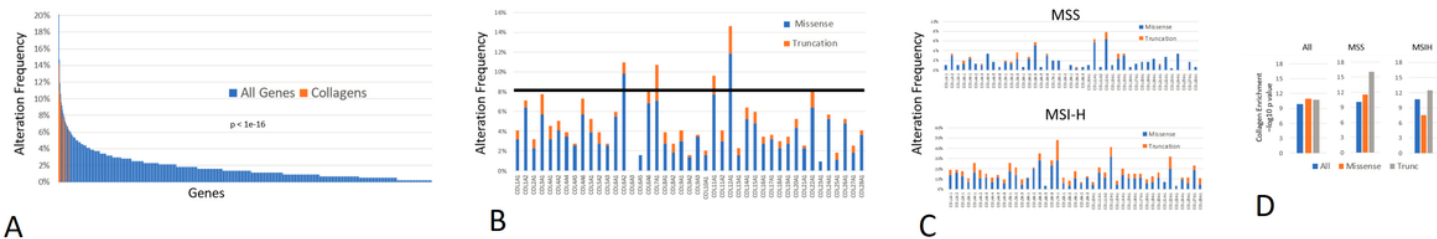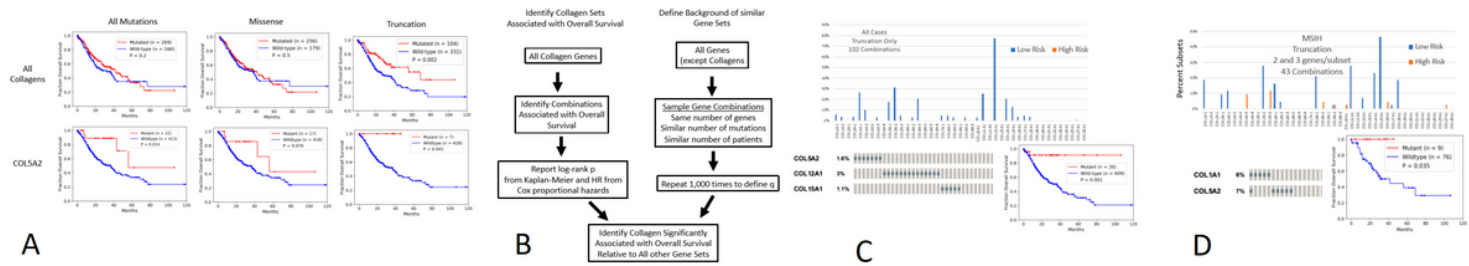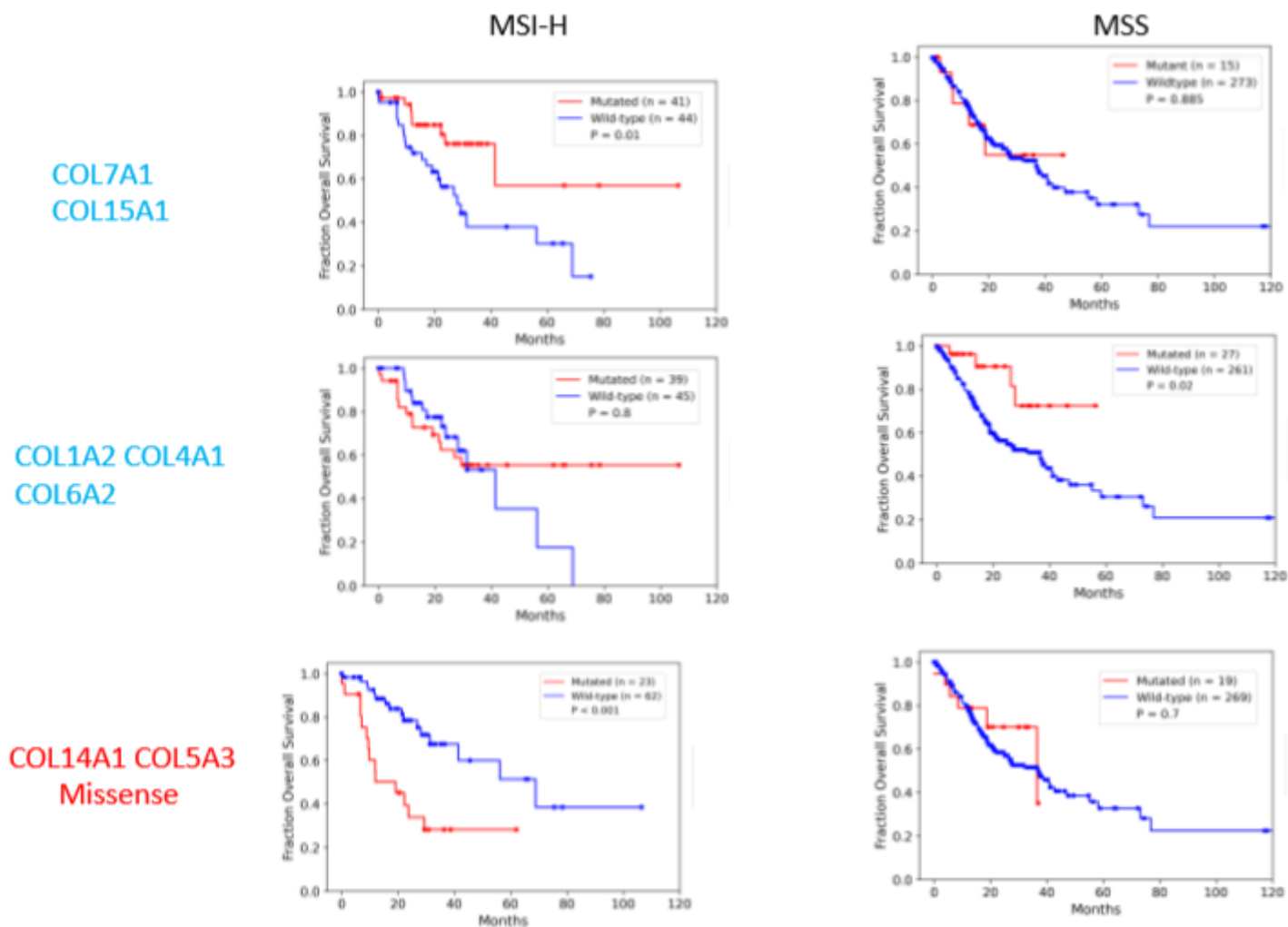
# Figures



Figure 1

Collagens are significantly mutated in stomach adenocarcinoma in the TCGA dataset. A. Distribution of alteration frequencies for collagen genes (orange) compared to all other genes (blue) in the TCGA STAD cohort. P-value determined by Wilcoxon rank test comparing the distribution of collagen genes relative to all other genes. B. Alteration frequencies for each collagen gene in all TCGA STAD cases. C. and in MSS, MSIH, and MSIL STAD cases. D. Kolmogorov-Smirnov moderated tests suggest that collagen genes as a group are significantly mutated compared to gene sets of similar size and length in the whole TCGA STAD cohort and in both MSS and MSIH tumors.
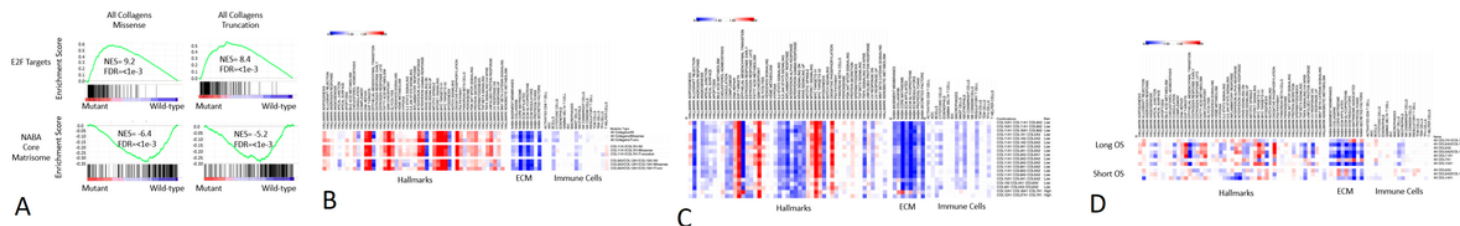
**Figure 2**

Identification of collagen genes mutations associated with overall survival. A. Patients with tumors that harbor at least one mutation in a collagen gene, have significantly better outcomes in the STAD TCGA cohort. Patients with tumors with at least one collagen mutation of the type indicated in red. Wild-type tumors in blue. Log-rank test p-values shown. Truncation mutations in any collagen gene were associated with better outcomes while nonsynonymous missense mutations were not associated with overall survival. Both missense and truncation mutations in COL5A2 were associated with longer overall survival. B. Schematic of approach to identify tumors with combinations of mutated collagens associated survival more significantly relative to background accounting for mutation rate, gene size and number of patients. C. Frequency of the inclusion of each collagen gene with a truncation mutation in a combination significantly associated with overall survival. A representative combination of collagen genes strongly associated with overall survival curve and the oncoprint. D. Identification of collagen genes with truncation mutations in MSIH tumors most strongly associated with overall survival. Frequency of the inclusion of each collagen in subsets consisting of 2 and 3 collagen genes with truncation only mutations in MSIH tumors.

MSI-H | MSS

COL7A1 COL15A1

COL1A2 COL4A1 COL6A2
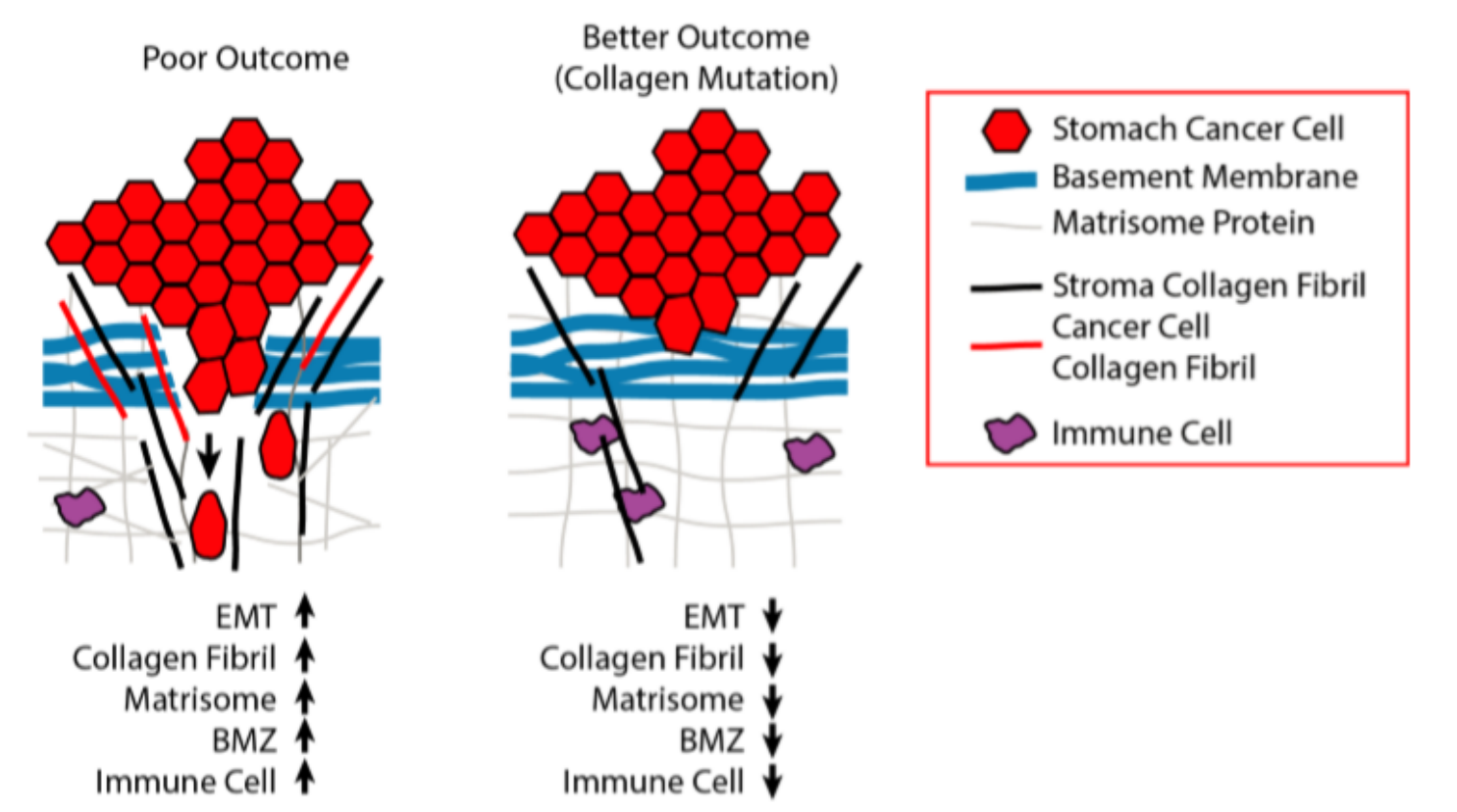
COL14A1 COL5A3 Missense

**Figure 3**

Specific collagen mutation combinations have context dependent association with overall survival in MSIH and MSS tumors. Kaplan-Meier survival analysis of representative collagen mutation combinations with differing patterns of association with overall survival in MSIH and MSS tumors. P-values determined by a log-rank test.



**Figure 4**

Tumors with collagen mutations have distinct expression of cancer hallmarks and tumor environments. A. Representative enrichment plots from pre-rank GSEA suggest upregulation of E2F regulated transcripts

and down-regulation of the expression of the matrisome in tumors with collagen mutations. TCGA stomach tumors were classified by collagen mutation status and pre-ranked GSEA revealed associations with the indicated gene set. B. Heat map of normalized enrichment scores of the cancer hallmark, immune cell, and NABA ECM gene sets. Red indicates higher expression in mutant tumors and blue indicates higher expression in wild-type tumors. Nonsignificant and modest enrichment scores between -1.5 and 1.5 are in white. In the full STAD TCGA cohort, tumors with any collagen mutation or with only mutations in COL7A1 or COL11A1 showed similar patterns. C. Heatmap of gene sets in MSS only tumors. D. Heatmaps of gene sets in MSIH only tumors reveal a more diverse pattern of enrichment. All heatmaps generated in Morpheus (54).



**Figure 5**

Model of impact of cancer cell secreted collagens on tumors. Collagens originate from either the cancer or stroma cells. Truncation and missense collagen mutants reorganize the tumor microenvironment decreasing multiple processes that increase drug sensitivity and reduce metastasis risk including reduced EMT, less local collagen around the cancer cells, a more disorganized collagen structure, and increased infiltration of cytotoxic immune cells and drugs.

# Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Tables.pdf
- AdditionalFile1.docx
- AdditionalFile2.xlsx
- AdditionalFile3.xlsx
- AdditionalFile4.docx
- AdditionalFile5.pdf