Molecular Characterization of Intestinal and Diffuse Types of Gastric Cancer

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Abstract

Background: Gastric adenocarcinomas are histologically categorized into intestinal (IS) and diffuse types (DS). TCGA categorization suggests that IS is more common in tumors arising via the chromosomal instability pathway and DS is more common in genomically stable tumors. However, molecular differences between these subtypes are not well understood.

Methods: Gastric adenocarcinomas were examined using NextGen sequencing (MiSeq platform on 47 genes or NextSeq on 592 genes), protein expression, and gene amplification techniques. For tumors sequenced with NextSeq, tumor mutational load (TML) was calculated based on somatic nonsynonymous missense mutations, and microsatellite instability (MSI) was evaluated on known MSI loci in target regions. Chi-square and t-tests were used for comparative analyses.

Results: In total, 296 gastric adenocarcinomas with annotated histology (DS [n = 181]; IS [n = 115]) were analyzed. Patients with DS were younger than those with IS (mean age: 58y [DS] vs. 67y [IS], p < 0.0001). The majority of patients with DS were female (51% [DS] vs. 35% [IS], p = 0.0051). Most frequently mutated genes in IS were *ARID1A* (70%), *TP53* (57%), *ATRX* (20%), *NF1* (15%); whereas the most frequent mutations in DS were *TP53* (45%), *ARID1A* (30%), *CDH1* (12%), *BAP1* (7%), and *RNF43* (5%). IS had a higher rate of *ARID1A* (70% vs. 30%, p=0.025), *ATRX* (20% vs. 0, p=0.028), *NF1* (15% vs. 0, p=0.005), *APC* (13% vs. 2%, p=0.007), *CDKN2A* (13% vs. 0, p=0.008) and *KRAS* (11% vs. 2%, p=0.017) mutations; whereas DS had a higher rate of *CDH1* (12% vs. 0, p = 0.0049). There was no difference in PD-L1 tumor expression (DS: 3%; IS: 9%). IS, when compared to DS, exhibited higher overexpression of TOP2A (95% vs. 56%, p < 0.0001), TS (67% vs. 30%, p < 0.0001), RRM1 (50% vs. 22%, p < 0.001), and Her2/neu (15% vs. 1%, p < 0.0001), as well as greater Her2 amplification (29% vs. 3%, p < 0.0001). MSI was seen in 5% of DS and 13% of IS, whereas high-TML is seen in 4% of DS and 8% of IS.

Conclusions: Significant molecular differences between IS and DS gastric adenocarcinomas were observed, a finding that indicates different carcinogenic pathways and biology, as well as potential differences in response to therapy. Low frequency mutations in several druggable genes may provide therapeutic options.

Background

- The vast majority of gastric cancers are adenocarcinomas, which can be subdivided into intestinal type (IS) and diffuse type (DS) according to the Lauren classification [1]
- In the TCGA molecular classification of gastric cancers, IS are included in the chromosomal instable group, whereas DS are comprised in the genomic stable group.
- DS are associated with young age, female gender, advanced stage disease, and poor prognosis compared to IS [2]. However, the molecular differences between these two subgroups are not well elucidated.
- For these reasons, we aim to characterize the molecular differences between IS and DS.

Methods

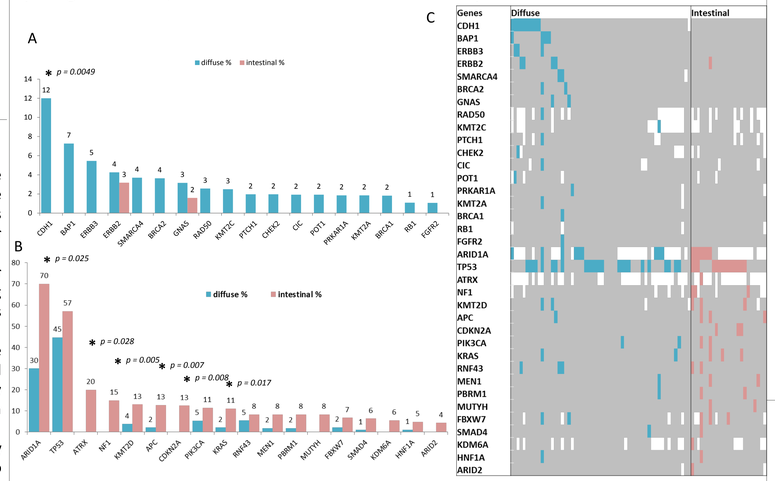
- Gastric tumor samples that underwent comprehensive genomic profiling by Caris Life Sciences (Phoenix, AZ) with clear indication as diffuse or intestinal histology were included; tumors with other or unclear histology were excluded our analysis; tumors with other or unclear histology were excluded. Chi-square test was performed for comparative analysis using SPSS.
- IHC was performed on FFPE sections of glass slides. Protein staining was scored for intensity (0 = no staining; 1+ = weak staining; 2+ = moderate staining; 3+ = strong staining) and staining percentage (0-100%) by pathologists. PD-L1 testing was performed using the SP142 anti-PD-L1 clone (Ventana, Tucson, AZ).
- NGS was performed on genomic DNA isolated from FFPE tumor samples using the NextSeq (592-genes)/Truseq platform (44-genes) (Illumina, Inc., San Diego, CA) . All variants were detected with greater than 99% confidence based on allele frequency and amplicon coverage, with an average sequencing depth of coverage of greater than 500 and an analytic sensitivity of 5%.
- MSI was examined by counting number of microsatellite loci that were altered by somatic insertion or deletion was counted for each sample. The threshold to determine MSI by NGS was determined to be 46 or more loci with insertions or deletions to generate a sensitivity of > 95% and specificity of > 99%.
- TML was estimated from 592 genes (1.4 megabases [MB] sequenced per tumor) by counting all non-synonymous missense mutations found per tumor that had not been previously described as germline alterations.

Results

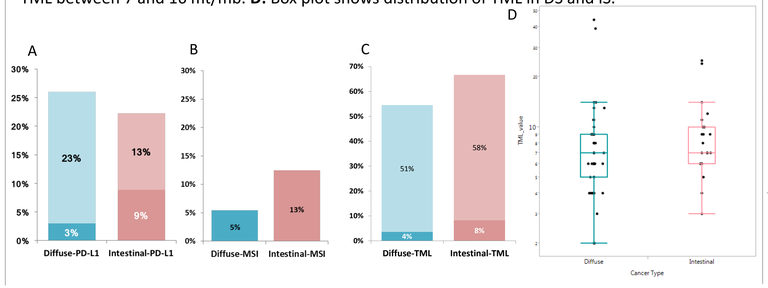
1. Patient Characteristics by histological type

Tumor Type		Diffuse	Intestinal	*P* value
Total N		181	115	
Gender	Female	93	40	0.0051
	Female %	51%	35%	
	Male	88	75	
	Male %	49%	65%	
Age	Average	57.8	67.4	<0.0001

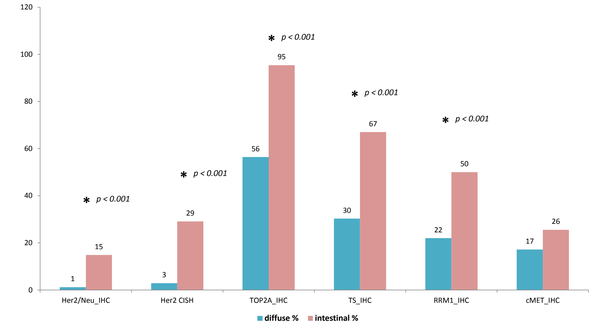
2: NextGen sequencing reveals significant difference between mutational landscapes of DS and IS. **A:** Gene mutations seen more frequently in DS than in IS. **B:** Gene mutations seen more frequently in IS than DS. For A & B: Y axis indicates the mutation frequency (%); data is from both TruSEQ and NextSEQ; significant differences are indicated by an asterisk with the p-value calculated by Chi-square test. **C:** Mutation pattern seen in DS and IS tumors tested with NextSEQ panel. Blue and Red indicates the pathogenic and presumed pathogenic mutations; grey indicates wild type or non-pathogenic variants, whereas blank indicates unavailable data points.



3: Immune checkpoint inhibitor-related biomarkers in DS (blue) and IS (red); A: PD-L1 expression. Dark color indicates expression level above 2+, 5%; light indicates expression level between 1+, 1%, and 2+, 5%. **B:** MSI-High prevalence tested by NextGen SEQ**. C:** Tumor mutational load (TML) tested by NextGen SEQ. Dark color indicates TML > 17 mutations (mt)/megabase (mb); light color indicates TML between 7 and 16 mt/mb. **D:** Box plot shows distribution of TML in DS and IS.



4: Immunohistochemistry (IHC) and CISH reveal significant differences between DS and IS. Y axis indicates the frequency (%) of positive expression and gene amplification seen. Significant differences are indicated by an asterisk with the p-values calculated by the Chi-Square test.



	Positive N	Total N	Diffuse %	Positive N	Total N	Intestinal %	p values
Her2/Neu_IHC	2	165	1%	15	101	15%	1.02E-05
Her2 CISH	2	69	3%	16	55	29%	3.9E-05
TOP2A_IHC	92	163	56%	83	87	95%	1.52E-10
TS_IHC	50	165	30%	63	94	67%	1.01E-08
RRM1_IHC	22	100	22%	30	60	50%	0.000251
cMET_IHC	11	64	17%	12	47	26%	NS

Conclusions

- Patients with DS tumors were significantly more likely to be female and younger compared to those with IS, reflecting the well-known distributions of Lauren's classification.
- IS had more MSI-H, TML-high (defined as TML > 17 mutations/megabase) status and PD-L1 overexpression compared to DS; however, none of these differences reached statistical significance.
- Significantly different mutation rates of *ARID1A*, *ATRX*, *NF1*, *APC*, *CDKN2A*, *KRAS* and *CDH1* and different expression rates of TOP2A, TS, RRM1, and HER2/neu, were observed between IS and DS, suggesting that different therapeutic strategies may be necessary depending on the tumor type.
- These findings provide insights into molecular differences between DS and IS, which may be useful in the development of novel tailored therapy for patients with gastric cancers.
- Low frequency mutations in several druggable genes may provide therapeutic options.

References

1. Cancer Genome Atlas Research. Nature, 2014. 513: 202-9.
2. Qiu et al. Journal of Translational Medicine 2013, 11:58