

Pathological Society Trainee Small Grant Scheme Report

Project title

Multi-omic passports of intestinal metaplasia in the human stomach

Award recipient details

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Background and aims

Gastric intestinal metaplasia (IM) is a precursor to intestinal-type gastric cancer (GC)¹, typically occurring in chronic *Helicobacter pylori* (HP) infection. Its clonal origins, epigenomic correlates and neoplastic potential remain largely unknown, however, with less than one percent of individuals with IM developing gastric cancer per person-year².

IM originates from mutant mucosal stem cells, which produce epithelia with a phenotype that may provide a survival advantage in the context of chronic mucosal inflammation³. Further molecular changes may lead to progression to malignancy³.

Epigenomic alterations are frequently observed in gastric cancers, as well as in non-neoplastic mucosa in chronic HP infection⁴. Molecular profiling of IM may help elucidate the pathogenesis of intestinal-type GC, as well as revealing biomarkers for risk-stratification of patients with IM, with potential implications for an evidence-based approach to endoscopic surveillance for early cancer. The aims of the project were:

1. Profile the epigenomic changes in gastric IM at clonal resolution
2. Identify shared alterations in gene expression between clonal patches of IM
3. Multi-omic comparison of genetic, epigenetic, and gene expression data to compile a 'molecular passport' of metaplastic tissue changes in the human stomach

Methods

Whole-exome sequencing was previously performed on clonal patches of IM obtained through laser-capture microdissection (LCM) from a cohort of 6 GC patients ($n = 42$ samples). A subset of these patches (2-3 patches per stomach) were co-sampled using LCM. Additional gastritis and muscle control samples were isolated using LCM. A separate control cohort was included for comparison from patients who had undergone bariatric gastric sleeve surgery, with histologically normal epithelium. DNA was extracted and bisulfite-converted, and samples were profiled on an Illumina epicArray850k. The Chip Analysis Methylation Pipeline (ChAMP) package in R was used for quality control, normalisation and differential methylation analysis. Analysis for methylation variability, differentially variable probes (DVPs) and differentially variable regions (DVRs) was performed with MissMethyl and DMRcate in R.

Results

$n = 32$ samples underwent methylation profiling. Principal component analysis showed highly distinct global clustering for IM versus gastritis and normal gastric samples. There was increased global methylation variability for IM versus other sampled tissue types. Differential methylation analysis identified 122,651 significantly differentially methylated probes (DMPs) for IM compared to gastritis, and 206,408 DMPs for IM

versus normal tissue. A number of DMPs were located in gene promoter regions with possible roles in intestinal phenotype switching and neoplastic progression. These included *CDX2*, a marker of intestinal differentiation, *RSPO2* and *RSPO3*, which have roles in the Wnt pathway, and *CDH1*, which encodes E-cadherin. 104 differentially methylated regions (DMRs) were found for IM versus gastritis and 92 for IM versus normal tissue, with a large degree of overlap (80%) between these phenotype comparisons. A small number of DMRs were located in cancer driver genes (*MLH1*, *APC*, *CD74*, *PRDM1*, and *SRC*). Methylation variability analysis identified 430 CpGs which were most variable in IM, of which 156 were in promoter regions.

Conclusions

This pilot study reveals early molecular changes associated with neoplastic progression in the chronically inflamed stomach. We have used genomic and epigenomic data to inform the development of a model of clonal expansion and pre-cancer progression. Further analysis is currently being undertaken on a second cohort of clinical gastric surveillance samples and neoplastic resections to further develop this.

How closely have the original aims been met?

This grant from the Pathological Society enabled epigenomic profiling of metaplastic tissue to be successfully completed. Ongoing work in the lab will explore gene expression profiling in gene sites identified during this pilot study.

Outputs

1. Tracing the genetic and epigenetic history of intestinal metaplasia in the chronically inflamed stoma to develop a model of pre-tumour progression. Waddingham, W.; Cross, W.; Ecker, S.; Williams, M.; Barmputis, P.; Shutkever, O.G. et al. Presented by WW at United European Gastroenterology Week 2020.

2. Tracing spatial clonal evolution of intestinal metaplasia in the chronically inflamed stomach – A quantitative model of pre-tumour progression. Waddingham, W.; Cross, W.; Shutkever, O.G.; Curtius, K.; Dawas, K.; Mohammadi, B. et al. Presented by WW at Digestive Disease Week 2020, Chicago, USA.

An abstract will also be presented at a future meeting of the Pathological Society.

References

1. Huang KK, Ramnarayanan K, Zhu F, Teh M, Yeoh KG, Tan P. Genomic and Epigenomic Profiling of High-Risk Intestinal Metaplasia Reveals Molecular Determinants of Progression to Gastric Cancer. *Cancer Cell*. 2018;33:137–50.
2. de Vries AC, van Grieken NCT, Looman CWN, Casparie MK, de Vries E, Meijer GA, et al. Gastric Cancer Risk in Patients With Premalignant Gastric Lesions: A Nationwide Cohort Study in the Netherlands. *Gastroenterology*. 2008;134(4):945–52.
3. Waddingham W, Graham D, Banks M, Jansen M. The evolving role of endoscopy in the diagnosis of premalignant gastric lesions. *F1000Research*. 2018;7.
4. Oliveira C, Suchi E, Calcagno Q. Epigenetic mechanisms in gastric cancer. *Futur Med*. 2012;4(3):279–94.