Gene expression of *NANOG* and *NANOGP8* in colorectal cancer

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Introduction

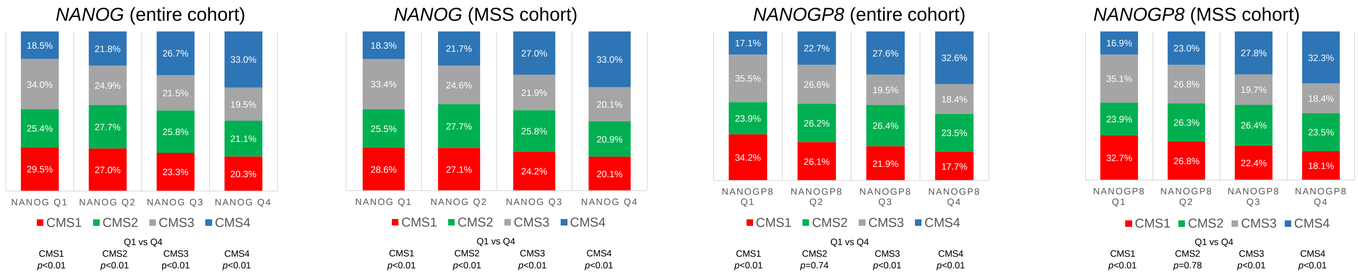
- The cancer stem cell (CSC) possesses self-renewal and multilineage differentiation potential, and believed to be responsible for resistance to chemotherapy and/or radiotherapy [1].
- NANOG is a pluripotency transcription factor that serves as a signaling hub in maintaining CSCs [2-3].
- Full-length NANOG protein is encoded by two paralogs of gene, namely *NANOG1* (generally referred as *NANOG*) and *NANOGP8* [4].
- NANOG mediates immune evasion through NANOG/TCL1A/AKT and NANOG/LC3B/EGFR axes, contributing to immune resistant phenotype [5-7].
- This study aimed to clarify molecular characters relating to gene expression levels of *NANOG* and *NANOGP8* in patients with colorectal cancer (CRC).

Methods

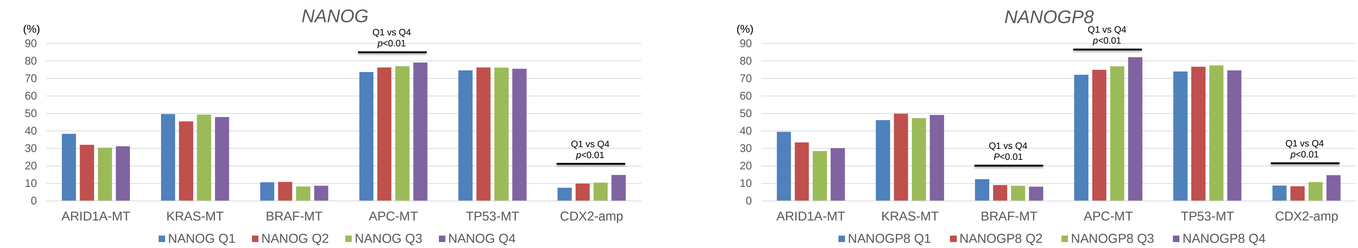
- Next-generation sequencing (NGS) and whole transcriptome sequencing (WTS) were performed on 7,604 CRC tumors submitted to Caris Life Sciences (Phoenix, AZ).
- Top quartile transcripts per million (TPM) for *NANOG* and *NANOGP8* expression were considered high (Q4); while bottom quartile was classified as low (Q1).
- Consensus molecular subtypes (CMS) were identified using WTS data.
- Microsatellite instability (MSI) / mismatch repair (MMR) status was tested with a combination of NGS, immunohistochemistry (IHC) and fragment analysis.
- Tumor mutational burden (TMB) was measured by counting all nonsynonymous missense mutations found per tumor [592 genes and 1.4 megabases (MB) sequenced/tumor]. The threshold to define TMB-high (TMB-H) was ≥10 mutations/MB.
- PD-L1 was tested by IHC (using SP142 antibody) and tumor proportion score >5% was regarded as PD-L1 positive.
- Cell infiltration in the tumor microenvironment (TME) was assessed using QuantiSEQ and MCP counter.
- Molecular profiles were compared between Q4 and Q1. CMS distribution, mutation/amplification profiles, and immunotherapy-related markers (IO markers: TMB, MSI/MMR status, and PD-L1 expression) were compared using Chi-Square or Fisher-Exact test. TME cell fractions were compared using non-parametric Kruskal-Wallis testing. Significance was determined by $p<0.05$ after adjusting for multiple comparison.

Results

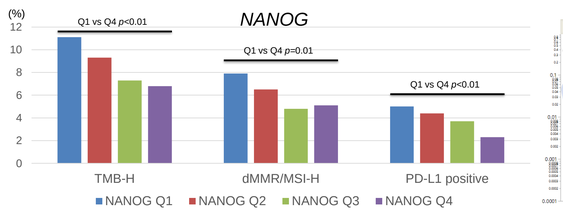
CMS distribution in *NANOG*/*NANOGP8* quartiles



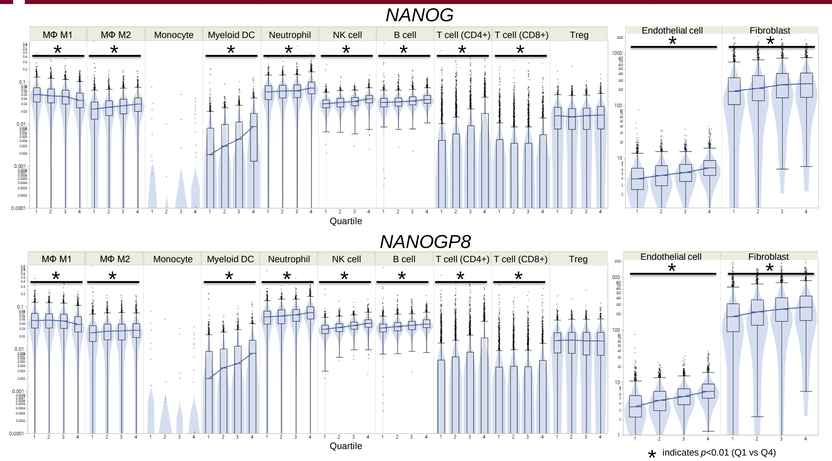
Mutation/amplification profiles in *NANOG*/*NANOGP8* quartiles



IO markers in *NANOG*/*NANOGP8* quartiles



TME components in *NANOG*/*NANOGP8* quartiles



Summary

- CMS1, CMS2, and CMS3 were negatively associated with *NANOG* TPM (Q1 > Q4, $p<0.01$) while CMS4 had a positive association (Q4 > Q1: 33% vs. 19%, $p<0.01$). Similarly, CMS1 and CMS3 were negatively associated with *NANOGP8* while CMS4 showed a positive association (33% vs. 17%). These associations were consistent in MSS cohort.
- *APC* mutations (*NANOG* Q4 vs. Q1: 79% vs. 74%; *NANOGP8*: 82% vs. 72%) and *CDX2* amplifications (15% vs. 8%; 15% vs. 9%) were more frequently observed in Q4 than Q1 of *NANOG* and *NANOGP8*.
- Positivity rates of TMB-H (*NANOG* Q4 vs. Q1: 7% vs. 11%; *NANOGP8* Q4 vs. Q1: 7% vs. 12%), dMMR/MSI-H (5% vs. 8%; 5% vs. 9%), and PD-L1 expression (2% vs 5%; 2% vs 6%) were all negatively associated with both genes' TPM.
- In the TME, abundance of macrophage M1 was significantly lower in Q4 while that of myeloid dendritic cells, neutrophils, NK cells, B cells, T cells (both CD4+ and CD8+), endothelial cells, and fibroblasts was higher in Q4 compared to Q1 of *NANOG* and *NANOGP8*.

Conclusions

CRC harboring high expression levels of *NANOG* and *NANOGP8* genes was enriched in CMS4 and had a possible association with alterations in the WNT pathway. These tumors had an inflammatory TME which may lead to resistance to immunotherapy. Further investigations including clinical outcome data are warranted to reveal the clinical implications of NANOG.

References

1. Medema JP. The New England journal of medicine 2017;377(9):888-90.
2. Iv Santaliz-Ruiz LE, et al. International Journal of Cancer 2014;135(12):2741-8.
3. Zhang J, et al. Oncogene 2013;32(37):4397-405.
4. Booth HA, et al. Genomics 2004;84(2):229-38.
5. Noh KH, et al. The Journal of clinical investigation 2012;122(11):4077-93.
6. Song KH, et al. Nature communications 2020;11(1):562.
7. Kim S, et al. Autophagy 2020:1-20.

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