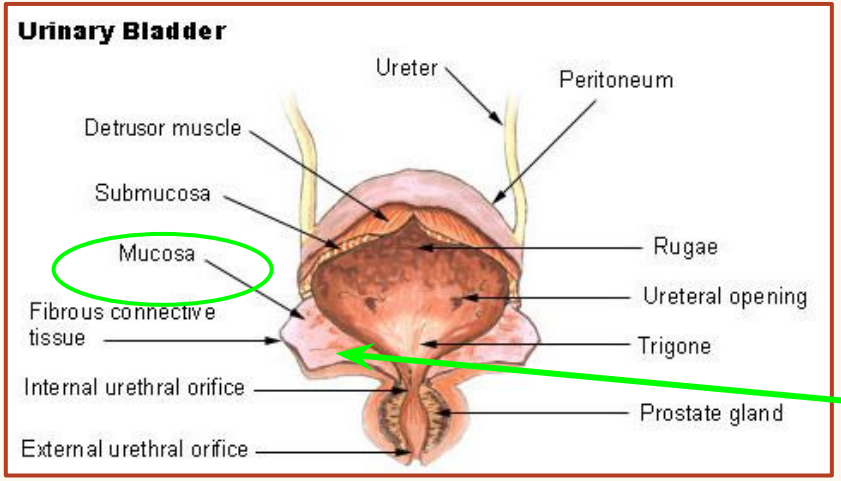
Bladder Cancer Differential Expression and CIBERSORT Analysis: Normal v. Precancerous

Team 1: Natalie, Fadwa, Stephen, and Austin
HIDS-7003 - Prof. Gusev, Dr. Bhuvaneshwar

Background



Focus: Mucosa layer – site of early pre-cancerous changes near bladder tumors

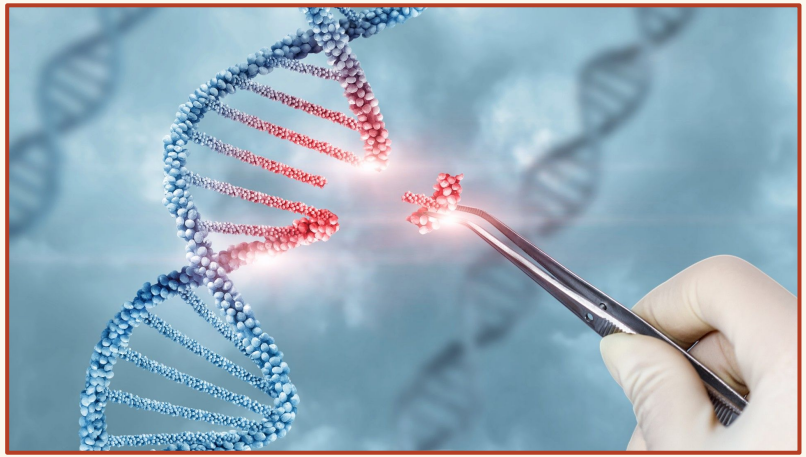
Bladder Cancer:

- abnormal cell growth in the bladder lining, often
- driven by genetic mutations and environmental risk factors.

Bladder Cancer Surrounding Mucosa (BCSM):

- Pre-cancerous tissue near bladder tumors.
- Genetic and molecular changes signal **early tumor development.**

Motivation



Why This Matters:

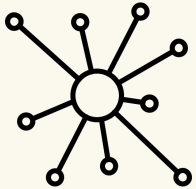
- Comparing **normal tissue** with BCSM helps uncover:
  - 🔬 **Biomarkers** for early detection of bladder cancer.
  - 🎯 **Therapeutic targets** to halt disease progression.
- This research can improve outcomes for high-risk patients.
- **Early detection** is critical to prevent pre-cancerous tissue from progressing into **invasive cancer.**

Research Question

Primary Objective:

- Compare **gene expression profiles** between normal bladder tissue and **Bladder Cancer Surrounding Mucosa (BCSM)** to uncover potential **molecular mechanisms** involved in bladder cancer development.

DEG Identification → Pathway Analysis → Immune Profiling → Integration of Results



Methodology

Sample

68 Patients
Nomal: 10 Patients
PreCancerous: 58 Patients

Data Source

Molecular Dataset: 43,148 Features
Clinical Dataset: 233 Patients

Data analysis

Step 1: Exploratory Data Analysis
Step 2: Gene Expression Analysis
Step 3: Systems Biology Analysis
Step 4: Immuno Oncology Analysis

Top Results

4 Significant Pathways
5 Top Immune Cell Types

CIBERSORT Results
Top 5 Immune Cell Types

Normal

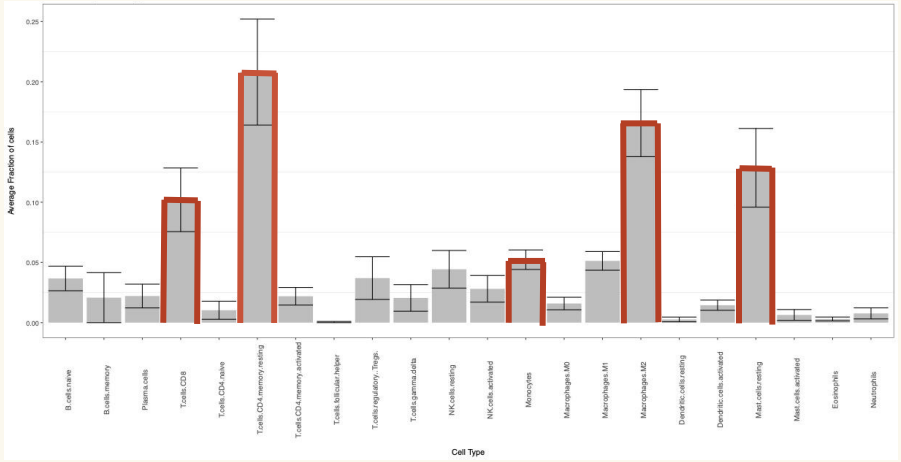
Cell type	Fraction average
T cells CD4 memory resting	0.2080746
Macrophages M2	0.16567027
Mast cells resting	0.12858561
T cells CD8	0.10200373
Monocytes	0.05224817

Precancerous

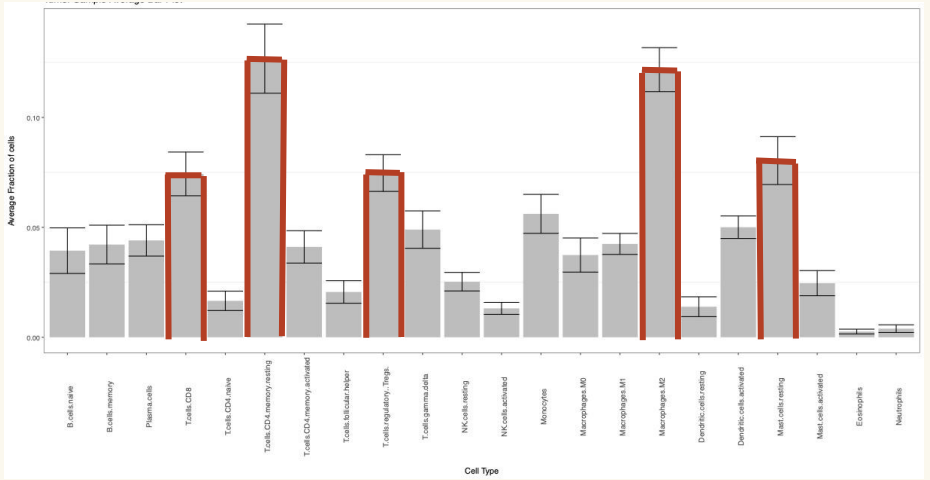
Cell type	Fraction average
T cells CD4 memory resting	0.1267045
Macrophages M2	0.12169419
Mast cells resting	0.08035757
T cells regulatory (Tregs)	0.074684
T cells CD8	0.07429097

CIBERSORT Results
Average Bar Plots

Normal

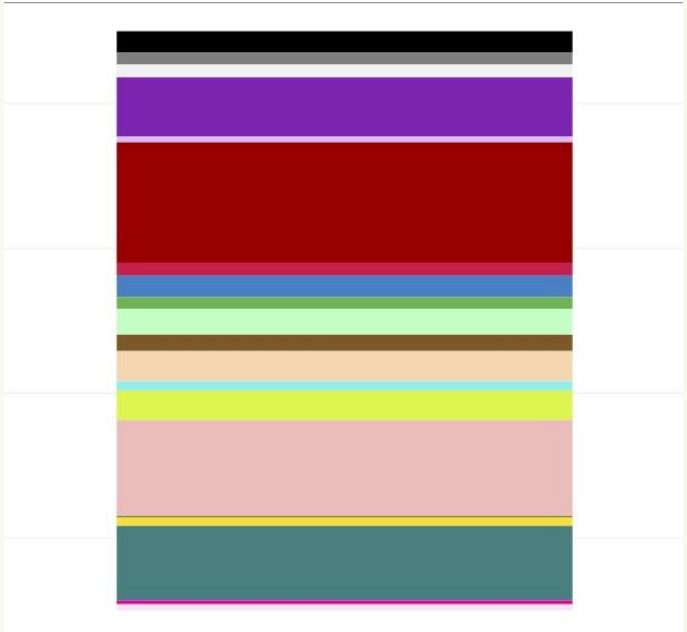


Precancerous

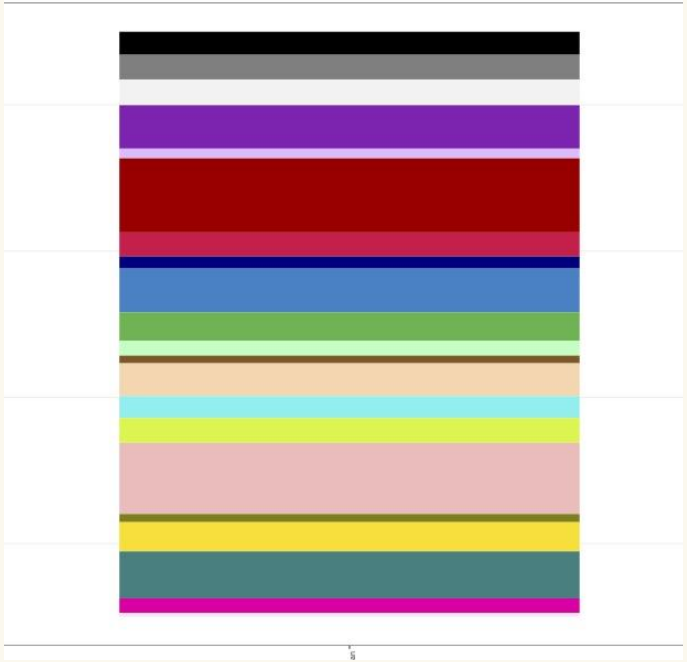


CIBERSORT Results
Stacked Bar Plots

Normal

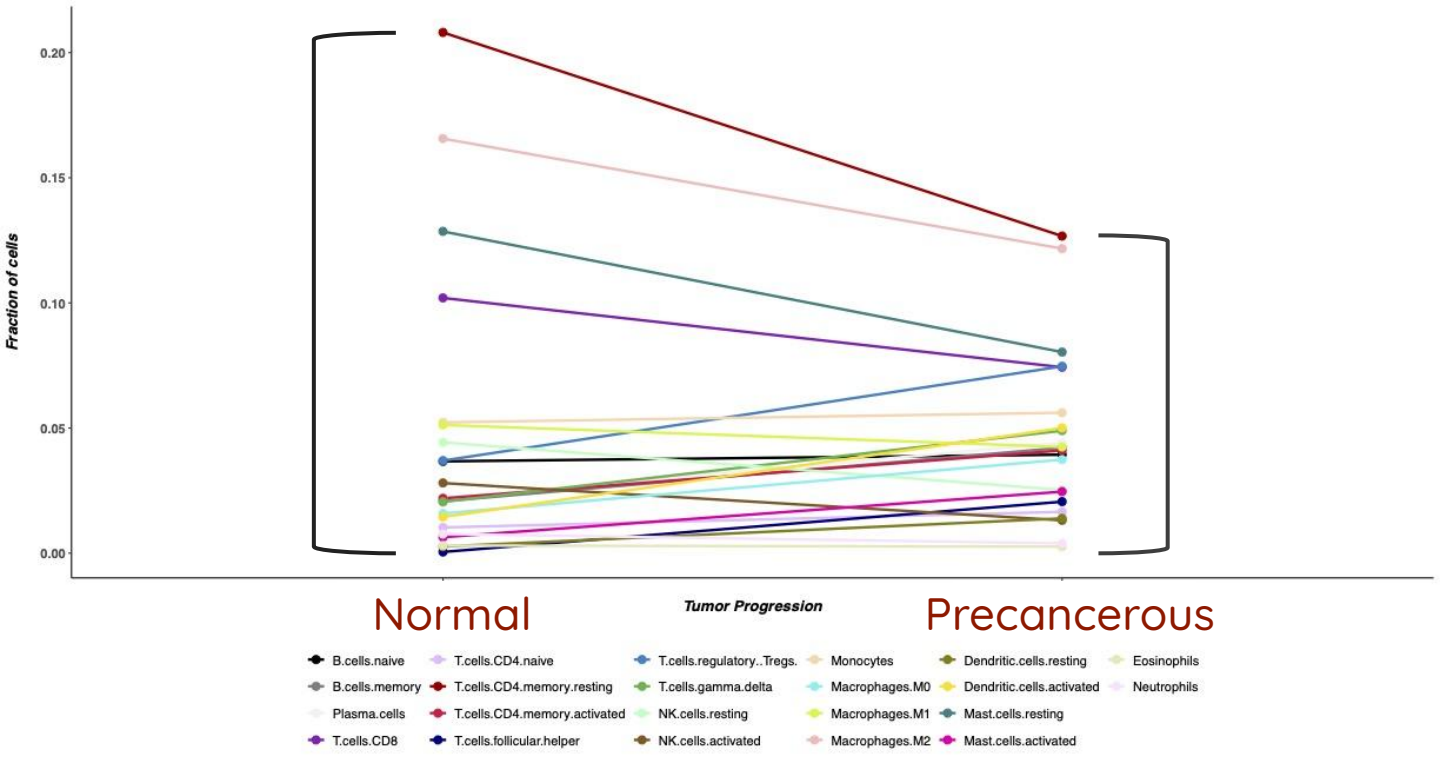


Precancerous

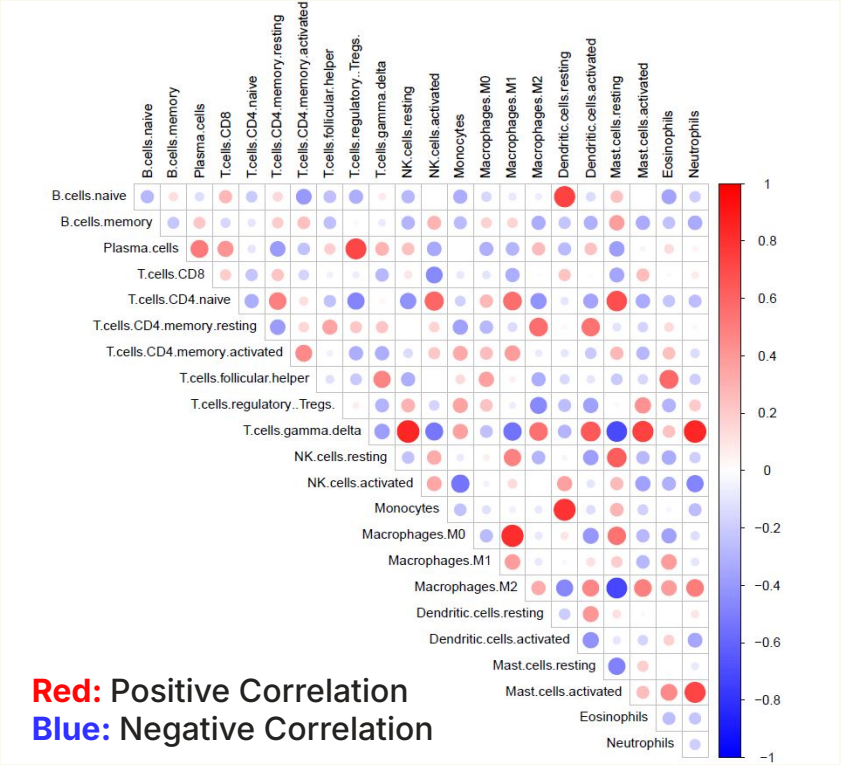


CIBERSORT Results

Line Plots: depicting change between samples



CIBERSORT Results



Correlation Plot: Detect any coordinated changes in more than one immune cell type

MHC Class II Presentation Pathway

Top Hit in Reactome

Combined score of 67.19

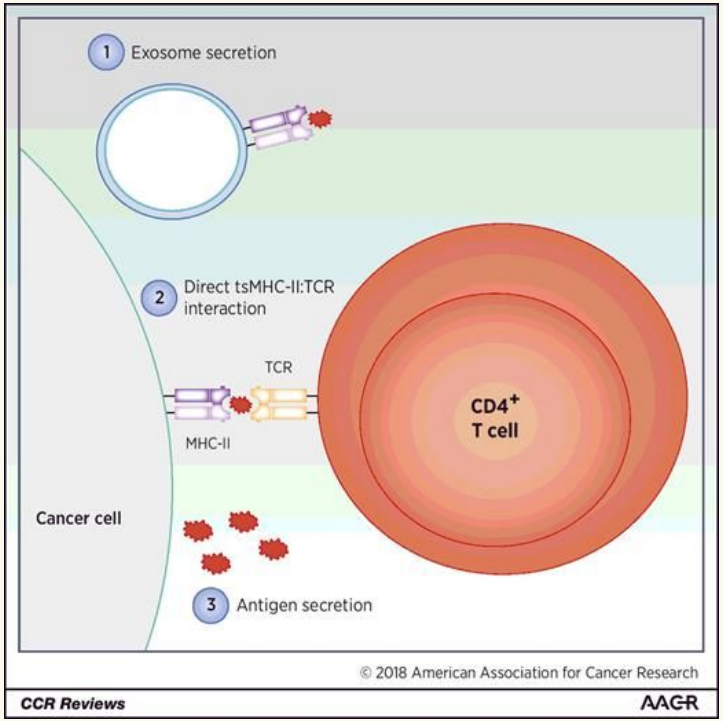
Functional link to CD4+ T cells

T cells CD4 memory resting changed the most from normal to precancerous

DEGS:

CENPE, KIF4A, CAPZA1, KIF23, KIF2C, KIF20A, KIF11, KIF15

MHC Class II Presentation Pathway
Overview



- **Major Histocompatibility Complex Molecules (MHC) Class II** [3]
- Bind and present antigenic peptides to **CD4+ T cells**
- Usually found on Professional Antigen-Presenting Cells (pAPCs), but tumors can present them as: **tumor-specific MHC Class II (tsMHC-II)** [1]

MHC Class II Presentation Pathway
Cancer Mechanism

Mechanism: Upstream

- tsMHC-II are induced by:
  - Inflammatory signals in tumor microenvironment
  - Aberrant epigenetic modifications or oncogenic signaling pathways

Mechanism: Downstream

- When CD4+ T cells bind to tsMHC-II, they can:
  - Activate and secrete cytokines
  - Support CD8+ T cells and B cells
  - Directly kill tumor cells
  - Modulate the tumor microenvironment
  - Promote the development of immune memory

[4]

MHC Class II Presentation Pathway
Bladder Cancer Relevance

BC cells are typically epithelial cells:

- Can present MHC-II in inflammatory environments
- **Lineage plasticity** - switch between different cell lineages or express markers from multiple lineages
- Allows tumor cells to express genes that are typically restricted to APCs

[2]

MHC Class II Presentation Pathway
Bladder Cancer Relevance

Bacillus Calmette-Guérin (BCG) Therapy for BC:

- Stimulates the immune system to attack bladder cancer cells
- Relies on tsMHC-II:

High expression = better prognosis

Lower expression = avoid detection by immune system

[2]

Toll-like Receptor Cascades

P-value of 0.33

Combined Score of 1.66

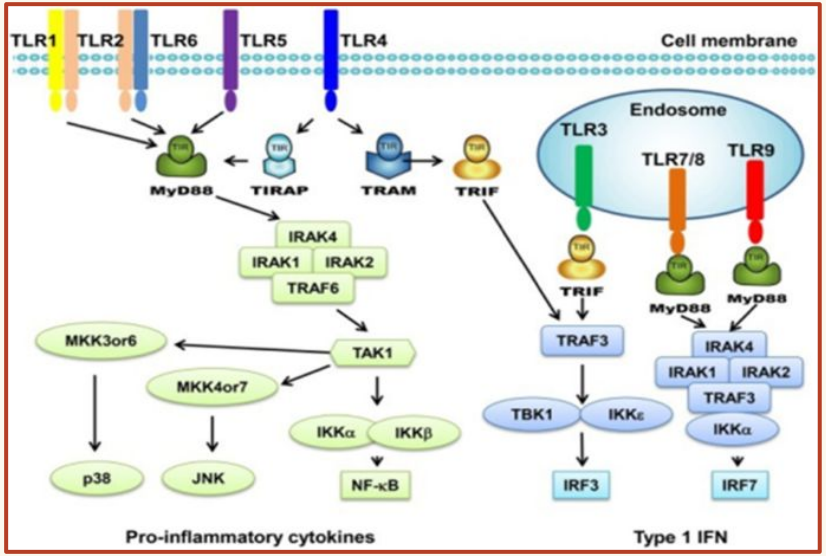
TLR sit on Dendritic Cell surface

mean proportion of activated DC's rose by .036 (significant increase) among immune cells when comparing normal to tumor cell types

DEGS:

TLR1, PIK3C3, TICAM1

Toll-like Receptor Cascades

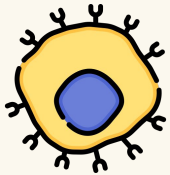


TLR steps

Pathogen Recognition

Cytokine Production

APC Maturation



T cell Activation

Toll-like Receptor Cascades

How the Tumor Microenvironment Causes DC Dysfunction

Tolerogenic DCs: Tumor microenvironment drives the development of tolerogenic DCs.

1. Promote **Tregs activation.**
2. Secrete **anti-inflammatory cytokines** (e.g., *TGF-β*, *IL-10*).

Increased Dendritic Cells in Pro-Tumorigenic Context

The immune system increases **DC activation** in response to early precancerous signals, but these DCs may become **functionally impaired** and pro-tumorigenic.

How DC Dysfunction Links to the TLR Pathway

TLR Suppression: Tolerogenic DCs **downregulate TLR signaling,** reducing cytokine production and immune activation against tumors.

Cell Cycle, Mitotic Pathway

First Result in Reactome

Combined score of 171.39

Process in most immune cells

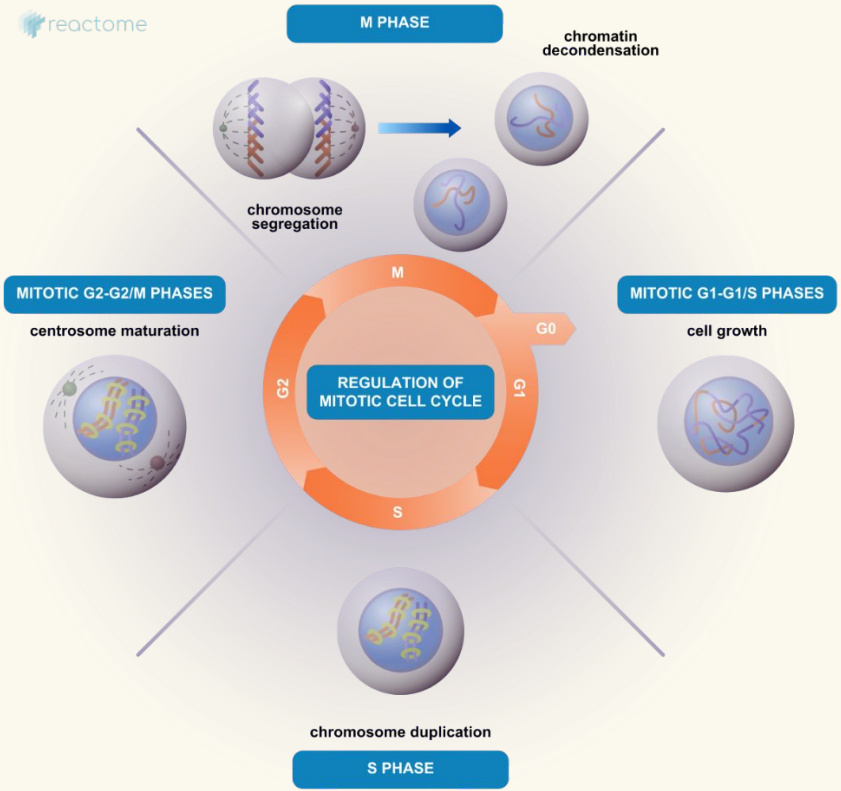
Example with Macrophages

DEGS:

TOP2A, CDCA5, NCAPG, **BUB1B**, HMMR, PKMYT1, **CENPA**, PSMA7, **AURKB**, AURKA, **CDC20**, CCNB2, E2F2, **KNTC1**, TK1, LCMT1, CDT1, GINS2, NUP210, UBE2C, **KIF23**, **CDC25C**, CDC25A, TPX2, **CENPE**, **CENPF**, ESPL1, **CENPL**, **CENPM**, **KIF2C**, **KIF20A**, **SPC25**

Cell Cycle, Mitotic Pathway

Overview



[6]

Cell Cycle, Mitotic Pathway
Macrophage Link

- Macrophages can take on tumor supporting characteristics after eating cancer cells
- Overabundance of cancer cells may lead tumor-enhancing macrophages to also develop rapidly
- Different types of cancer may have different results in these interactions

[7]

Cell Cycle, Mitotic Pathway
Bladder Cancer Relevance

- Uncontrolled division of cells results in cancer
- 2 major proteins: Cyclins and Cyclin-Dependent Kinases (CDKs)
- Leads to tumor suppressing functions not working later on

[8]

Cell Cycle, Mitotic Pathway
Bladder Cancer Relevance

- Mitotic activity related to frequency of **recurrence** and **progression**
- **High** and **low** activity both can have an effect

[9]

RHO GTPase Effectors Pathway

Top Result in Reactome

P-Value of **3.02^{e-7}**

Combined score of **79.10**

Macrophage M2 Polarization

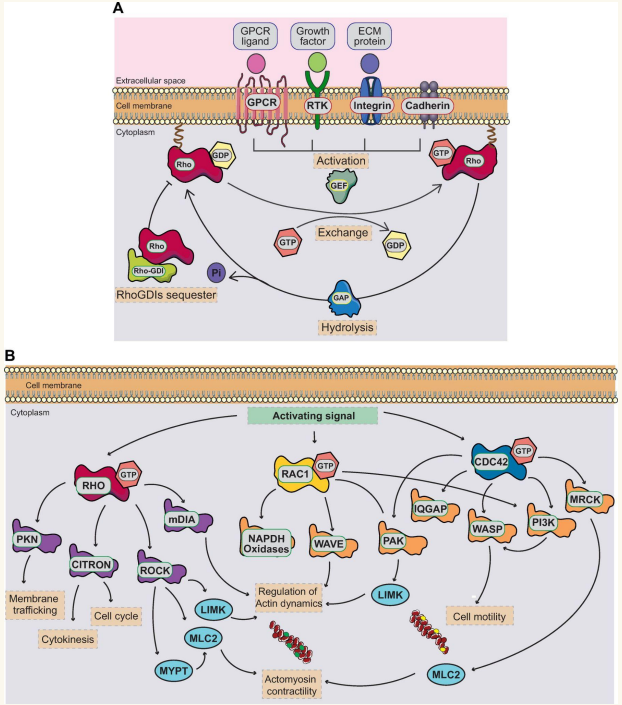
Average fraction **decreased by -27%** from normal to precancerous

DEGS:

KIF14, BUB1B, CDC25C, IQGAP3, CENPA, AURKB, **CDC20**, CENPE, CENPF, PRC1, CENPL, CENPM, KIF2C, KNTC1, PIK3C3, SPC25

RHO GTPase Effectors Pathway

Overview



Regulate cytoskeleton dynamics, cell shape, motility, cycle, survival, growth, and gene expression.

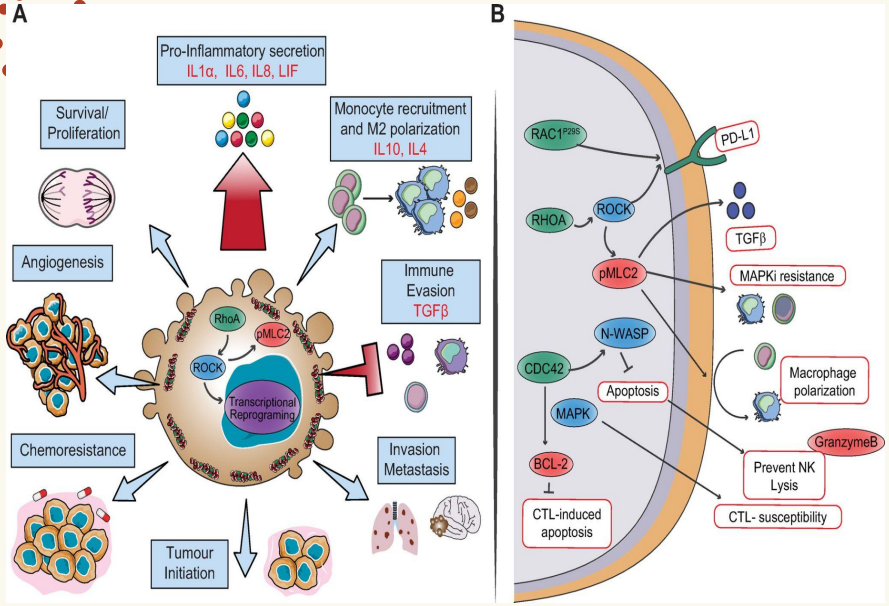
20 RHO GTPase, classified into:

- RHO
- RAC
- CDC42

[#]

RHO GTPase Effectors Pathway

Overview



Contributes to Hallmarks of Cancer

- **RHOA** is a key Rho GTPase involved in cancer cell survival in the bloodstream
- High Rho kinase (ROCK) activity is a key feature of highly aggressive cancers
- ROCK drives high TGFβ secretion

[#]

RHO GTPase Effectors Pathway

Interaction with the immune microenvironment

ROCK-Myosin II activity drives **M2 macrophage polarization**, an immunosuppressive phenotype.

ROCK **reduces CD8+ infiltration** into tumors, suppressing cytotoxicity.

TGFβ signaling induces Tregs via FOXP3 expression, enhancing immunosuppression.

Immune evasion driven by TGFβ induces DC apoptosis, and impedes DC migration

[#]

RHO GTPase Effectors Pathway
Bladder Cancer Relevance

- RHOA has been found mutated in bladder cancer
- Protein levels of RHOA, RHOC and CDC42 were significantly higher in UCC vs. normal tissue
- High CDC42 mRNA levels correlated with worse overall survival
- RHOA-ROCK-Myosin II increased resistance to membrane disruption in bladder cancer cells
- Pak1, a downstream effectors of Rac1, is associated with invasiveness, metastases, and recurrence of urothelial carcinoma

RHO GTPase Effectors Pathway
Therapeutic Opportunities

- Targeting the ROCK pathway with fasudil could inhibit urothelial tumour cell proliferation

[9]

Conclusion

Graphs:

Key changes in immune cell proportions suggest a disrupted immune landscape

MHC Class II:

Impaired antigen presentation may weaken immune responses and contribute to BC recurrence

TLR Cascade

Impaired dendritic cells suppressed immune activation, allowing the tumor to evade the immune system

Cell Cycle, Mitotic

Abnormal mitotic activity associated with tumor progression and recurrence

RHO GTPase

Plays a pivotal role in regulating both tumor progression and the immune microenvironment in BC.

Future Improvements

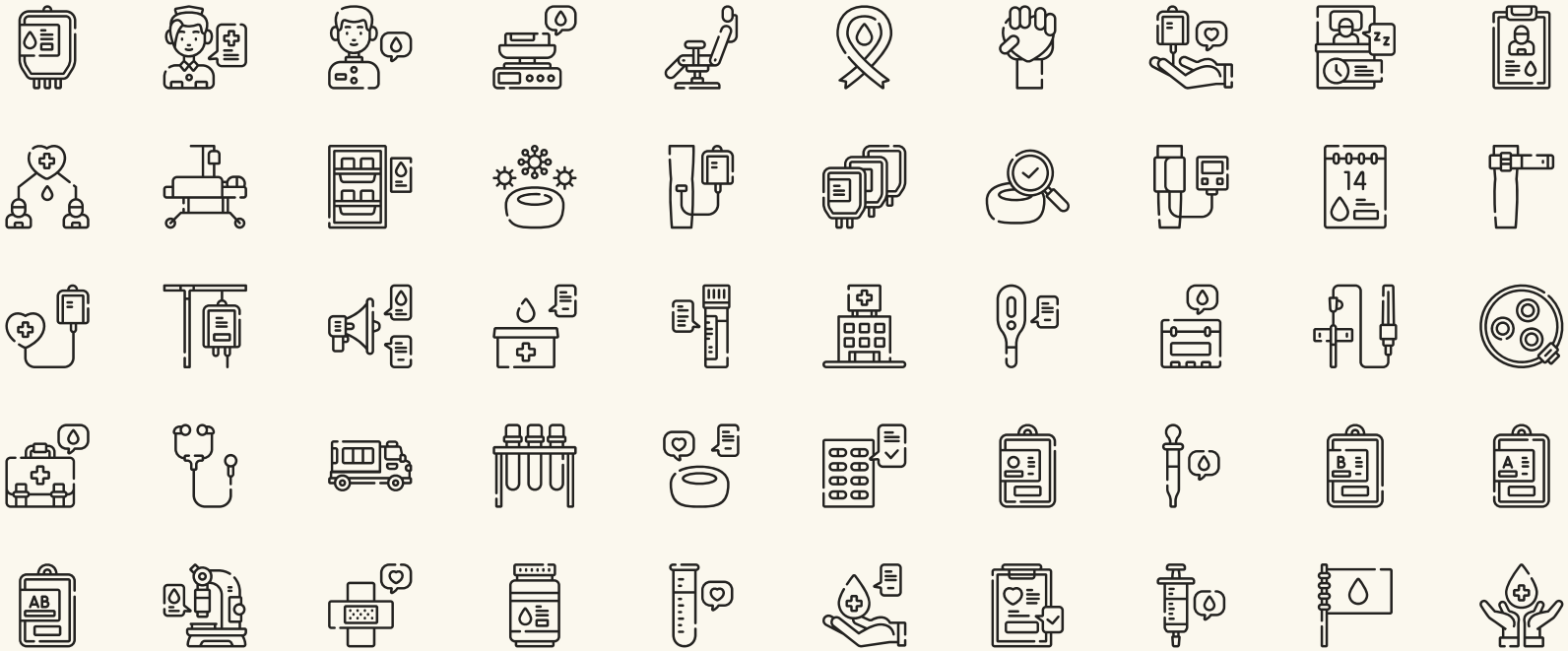
- Longitudinal Analysis of pathways
- Further analysis of DEGs
- Look into other immune cell-related pathways

Thanks

Do you have any questions?

Please keep this slide for attribution

Icon pack



Alternative resources

Here’s an assortment of alternative resources whose style fits that of this template:

Vectors:

- Flat design blue dots background

References

1. Margaret L. Axelrod, Rebecca S. Cook, Douglas B. Johnson, Justin M. Balko; Biological Consequences of MHC-II Expression by Tumor Cells in Cancer. *Clin Cancer Res* 15 April 2019; 25 (8): 2392–2402. https://doi.org/10.1158/1078-0432.CCR-18-3200
2. Yi R, Hong S, Zhang Y, Lin A, Ying H, Zou W, Wang Q, Wei T, Cheng Q, Zhu W, Luo P and Zhang J (2022) MHC-II Signature Correlates With Anti-Tumor Immunity and Predicts anti-PD-L1 Response of Bladder Cancer. *Front. Cell Dev. Biol.* 10:757137. doi: 10.3389/fcell.2022.757137
3. Roche PA, Furuta K. The ins and outs of MHC class II-mediated antigen processing and presentation. *Nat Rev Immunol*. 2015;15(4):203-216. doi:10.1038/nri3818
4. Sfakianos, J.P., Daza, J., Hu, Y. *et al.* Epithelial plasticity can generate multi-lineage phenotypes in human and murine bladder cancers. *Nat Commun* 11, 2540 (2020). https://doi.org/10.1038/s41467-020-16162-3
5. Ma Y, Shurin GV, Zhu Peiyuan, Shurin MR. Dendritic Cells in the Cancer Microenvironment. *Journal of Cancer*. 2013;4(1):36-44. doi:https://doi.org/10.7150/jca.5046
6. National Center for Biotechnology Information. PubChem Pathway Summary for Pathway R-HSA-69278, Cell Cycle, Mitotic, Source: Reactome. https://pubchem.ncbi.nlm.nih.gov/pathway/Reactome:R-HSA-69278. Accessed Dec. 18, 2024.
7. Zhang Y, Zhou N, Yu X, et al. Tumacrophage: macrophages transformed into tumor stem-like cells by virulent genetic material from tumor cells. Oncotarget. 2017;8(47):82326-82343. Published 2017 Jul 18. doi:10.18632/oncotarget.19320
8. Mercadante AA, Kasi A. Genetics, Cancer Cell Cycle Phases. [Updated 2023 Aug 14]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK563158/
9. Michael Zaleski, Augustyna Gogoj, Vonn Walter, et al. Mitotic activity in noninvasive papillary urothelial carcinoma: its value in predicting tumor recurrence and comparison with the contemporary 2-tier grading system, Human Pathology, Volume 84, 2019, Pages 275-282, ISSN 0046-8177, https://doi.org/10.1016/j.humpath.2018.10.008. (https://www.sciencedirect.com/science/article/pii/S0046817718303952)
10. Volanis, D., et al. (2011). Expression profile of Rho kinases in urinary bladder cancer. *Journal of BUON*, 16(3), 511–521. Retrieved from https://www.spandidos-publications.com/var/spand/publication_585.pdf
11. Crosas-Molist, E., et al. (2021). Rho GTPase signaling in cancer progression and dissemination. *Physiological Reviews*, 101(4), 1765–1799. https://doi.org/10.1152/physrev.00045.2020
12. Woldu, S. L., et al. (2018). The Rho GTPase signalling pathway in urothelial carcinoma. *Nature Reviews Urology, 15*(2), 83–91. https://doi.org/10.1038/nrurol.2017.184