The Dynamic Role of LGR5 in EGFR-expressing Cancers

The Presentation Contains Three (3) Distinct Chapters

The Battle Against Epithelial Cancers

- Burden and hallmarks
- Limitations of EGFR therapy
- Resistance mechanisms

Pathogenic Plasticity

- Hallmark hierarchy
- Natural plasticity
- Pathogenic role of LGR5

LGR5, the Moving Target

- LGR5 and EGFR therapy
- LGR5 recycling dynamics
- Expression of LGR5 during metastasis

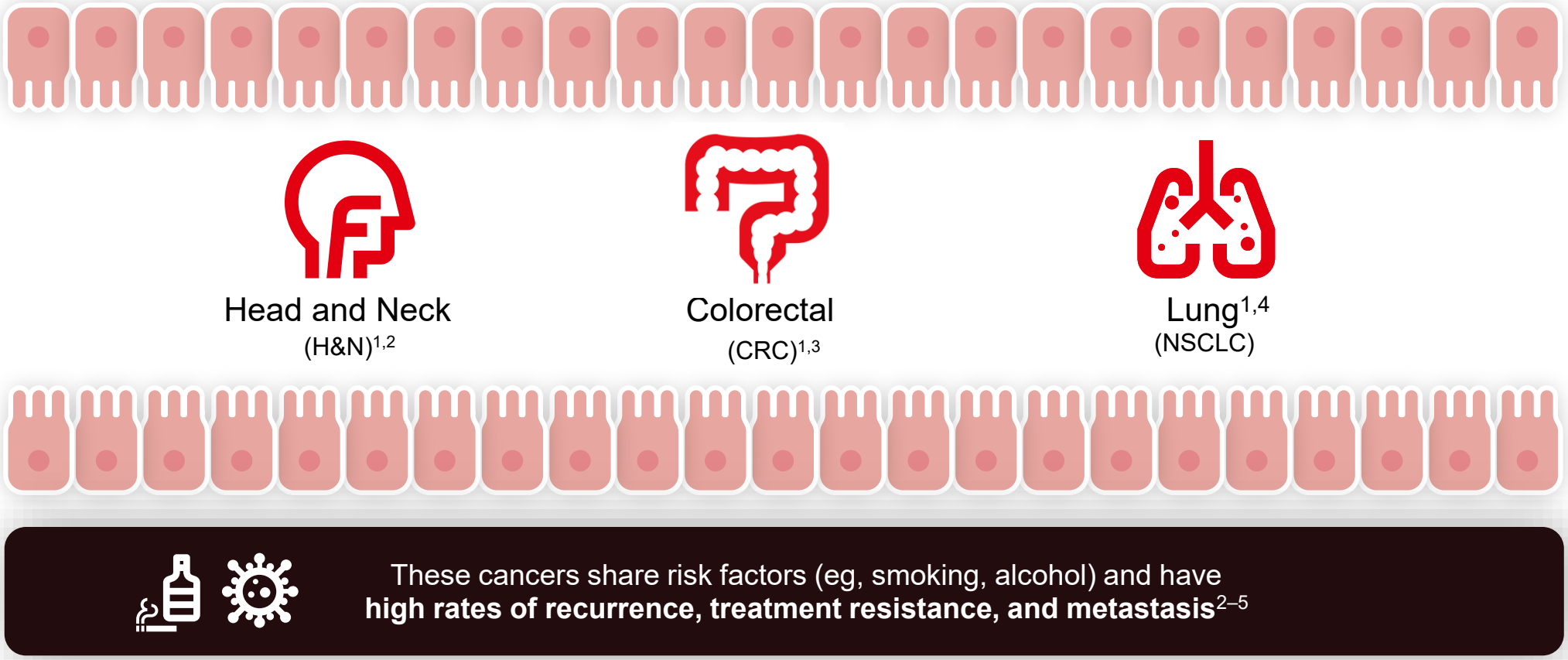
EGFR, epidermal growth factor receptor; LGR5, leucine-rich repeat-containing G-protein coupled receptor 5.

Chapter 1

The Battle Against Epithelial Cancers

Epithelial Cancers (Carcinomas) Arise From Cells That Line the Surfaces and Organs of the Body[1–4]

H&N, CRC, and NSCLC are examples of epithelial cancers



NSCLC, non-small cell lung cancer.

1. National Cancer Institute. Epithelial tissue. https://training.seer.cancer.gov/anatomy/cells_tissues_membranes/tissues/epithelial.html. Accessed February 2025; 2. Ionna F et al. *Cancers (Basel).* 2021;13(10):2371; 3. Kanwar SS et al. *World J Gastrointest Pathophysiol*. 2012;3(1):1-9; 4. Tahayneh K et al. *J Clin Med*. 2025;14(3):1025; 5. van Zutphen M et al. *Curr Colorectal Cancer Rep.* 2017;13:370-401.

H&N, CRC, and Lung Cancers Are Common in the US and Claim the Lives of Thousands of People Every Year[1]

US Cancer Estimates (2025)

	Deaths	New cases
Head and neck[a]	16,680	72,680
Lung and Bronchus	124,730	226,650
Colon and rectal	52,900	154,270

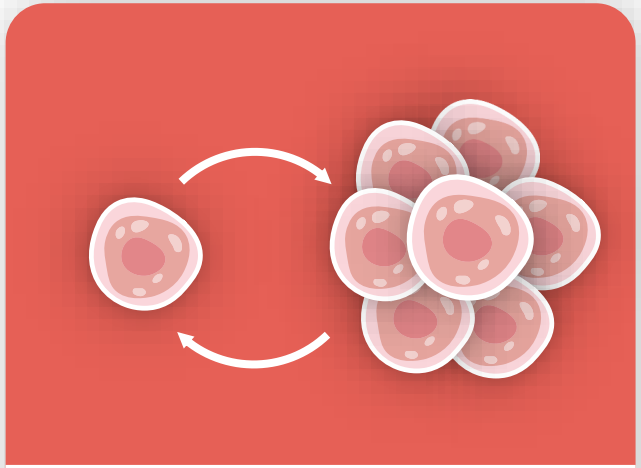
~200,000 deaths

~450,000 new cases

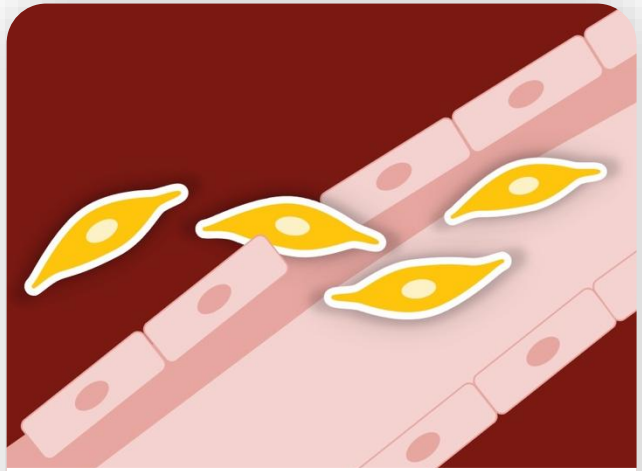
[a]Oral cavity, pharynx, larynx.

1. Siegel RL et al. *CA Cancer J Clin*. 2025;75(1):10-45.

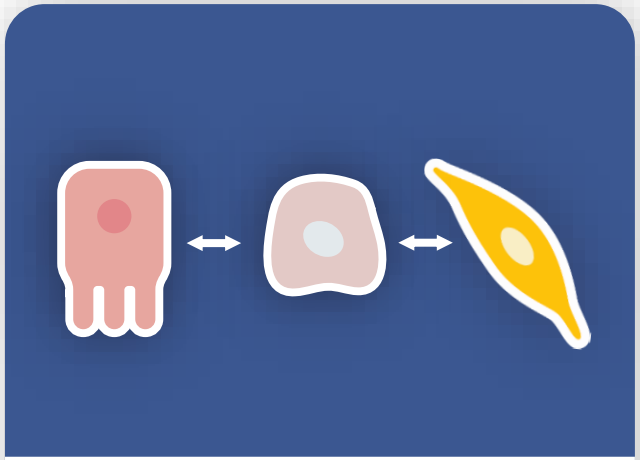
Several Cancer Hallmarks Contribute to the Pathogenesis of Epithelial-Derived Cancers[1–5]



Proliferation and cellular dormancy[1,5]



Invasion and metastasis[1–3]



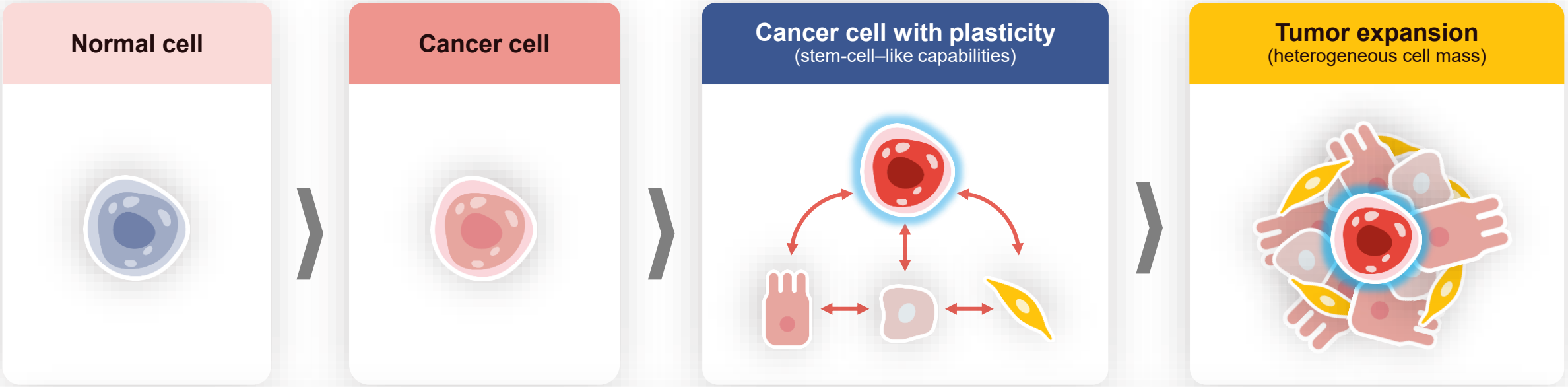
Cell plasticity and reprogramming[1,3]

> “There is increasing evidence that unlocking the normally restricted capability of plasticity is a critical component of cancer pathogenesis[1]

1. Hanahan D. *Cancer Discov*. 2022;12(1):31-46; 2. Hanahan D, Weinberg RA. *Cell*. 2011;144(5):646-674; 3. Mäkitie AA et al. *Head Neck*. 2019;41(10):3712-3718;
4. Li J et al. *Genes Dev.* 2021;35(11-12):787-820; 5. Song Y et al. *Front Bioeng Biotechnol*. 2023;11:1132940.

Plasticity and Cellular Reprogramming Give Cancer Enhanced Capabilities[1,2]

Cancer cells originating from a normal, fully differentiated cell can obtain plasticity, **stem-cell–like capabilities that allow cancer cells to change behavior and phenotype[1]**



Cancer cells with plasticity can self-renew and transition into other cell types, **enabling tumor growth, treatment resistance, and metastasis**[3–6]

1. Hanahan D. *Cancer Discov*. 2022;12(1):31-46; 2. Li J et al. *Genes Dev*. 2021;35(11-12):787-820; 3. Xu Q et al. *Oncotarget.* 2017;8(6):9557-9571; 4. Major AG et al. *Stem Cells Int*. 2013;2013:319489; 5. Shimokawa M et al. *Nature*. 2017;545(7653):187-192; 6. Cortina C et al. *EMBO Mol Med*. 2017;9(7):869-879.

An Example of Cancer Cell Plasticity Is Treatment Resistance to EGFR-targeted Therapy[1,2]

EGFR overexpression is common in H&N, CRC, and NSCLC[3–5]

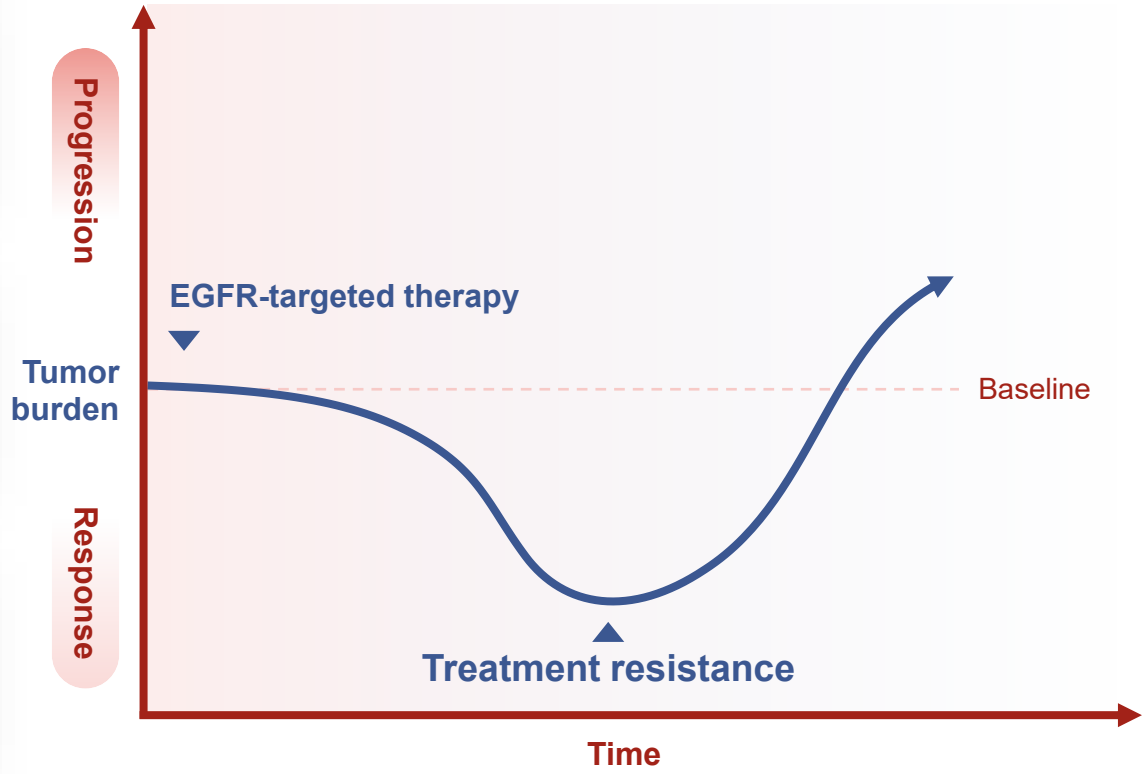
Implicated in tumor growth, invasion, and resistance

EGFR-targeted therapies can block receptor signaling[3–6]

Cancer commonly develops resistance

Resistance occurs through multiple mechanisms[1,2,7]

Epithelial-to-mesenchymal transition (EMT)

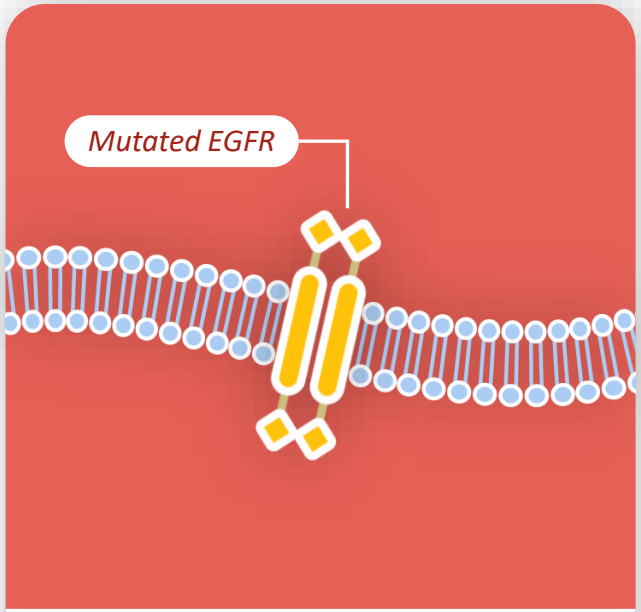


The figure is hypothetical and does not reflect data from a specific clinical study

EGFR, epidermal growth factor receptor.

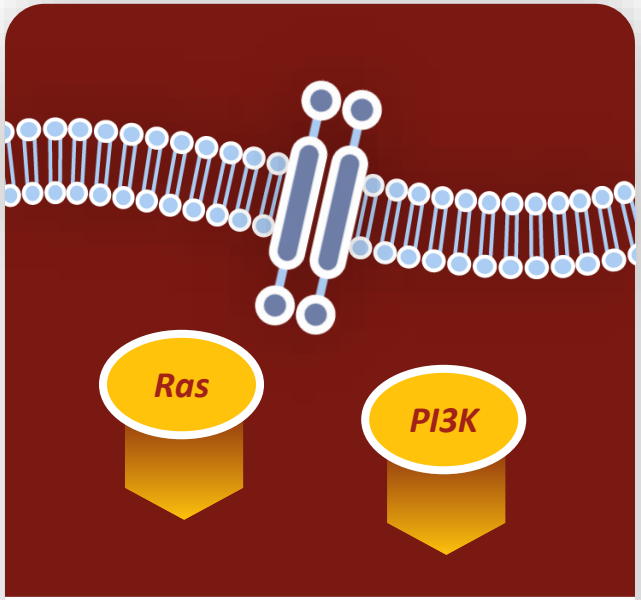
1. Chhouri H, et al. *Cancers (Basel).* 202020;44(6):1267-1282; 2. Zhou J, et al. *J Exp Clin Cancer Res*. 2021;40(1):328; 3. Halder S et al. *Expert Opin Ther Targets*. 2023;27(4-5):305-324; 4. London M, Gallo E. *Cell Biol Int*. 2020;44(6):1267-1282; 5. Xu MJ et al. *Cancer Metastasis Rev.* 2017;36(3):463-473; 6. Liu B et al. *Signal Transduct Target Ther*. 2024;9(1):175; 7. Kobayashi K. *J Respir*. 2023;3(4):223-236.

Epithelial-to-Mesenchymal Transition (EMT) Is an Established Resistance Mechanism to EGFR-targeted Therapy[1,2]



EGFR Mutations

Mutations interfere with treatment binding site



Downstream Mutations

Activation of signaling pathways that bypass EGFR

Cell Transition

Transition to another cell lineage (epithelial → mesenchymal)[a]

[a]EMT is the process by which cells lose their epithelial characteristics (eg, cell-to-cell-adhesion) and acquire mesenchymal traits (eg, motility).[3]

1. Chhouri H et al. *Cancers (Basel)*. 2023;15(2):504; 2. Zhou J et al. *J Exp Clin Cancer Res*. 2021;40(1):328; 3. Hanahan D. *Cancer Discov*. 2022;12(1):31-46.

The Battle Against Epithelial Cancers

Key takeaways

1. **Epithelial cancers** (eg, H&N, CRC, lung) are common and have a **poor prognosis**, despite an established treatment target (eg, EGFR)[1–3]

2. Multiple hallmarks contribute to the pathogenesis of epithelial tumors and **plasticity has emerged as a critical factor in cancer pathogenesis**[4]

3. **Plasticity**, in the form of EMT, is an established **resistance mechanism** to EGFR-targeted therapy[5,6]

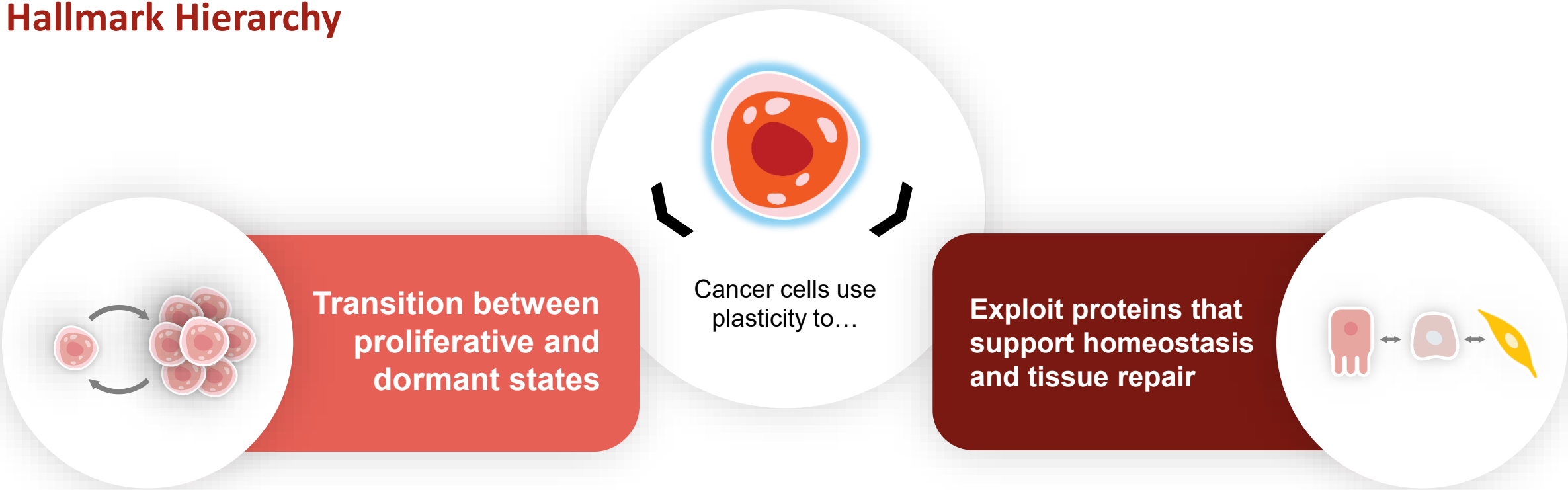
1. Siegel RL et al. *CA Cancer J Clin*. 2025;75(1):10-45; 2. Halder S et al. *Expert Opin Ther Targets*. 2023;27(4-5):305-324; 3. Kobayashi K. *J. Respir*. 2023;3:223-236; 4. Hanahan D. *Cancer Discov*. 2022;12(1):31-46; 5. Chhouri H, et al. *Cancers (Basel)*. 2023;15(2):504; 6. Zhou J, et al. *J Exp Clin Cancer Res*. 2021;40(1):328.

Chapter 2

Pathogenic Plasticity

Cell Plasticity Drives Epithelial Cancer Progression by Orchestrating the Action of Other Hallmarks[1]

Hallmark Hierarchy

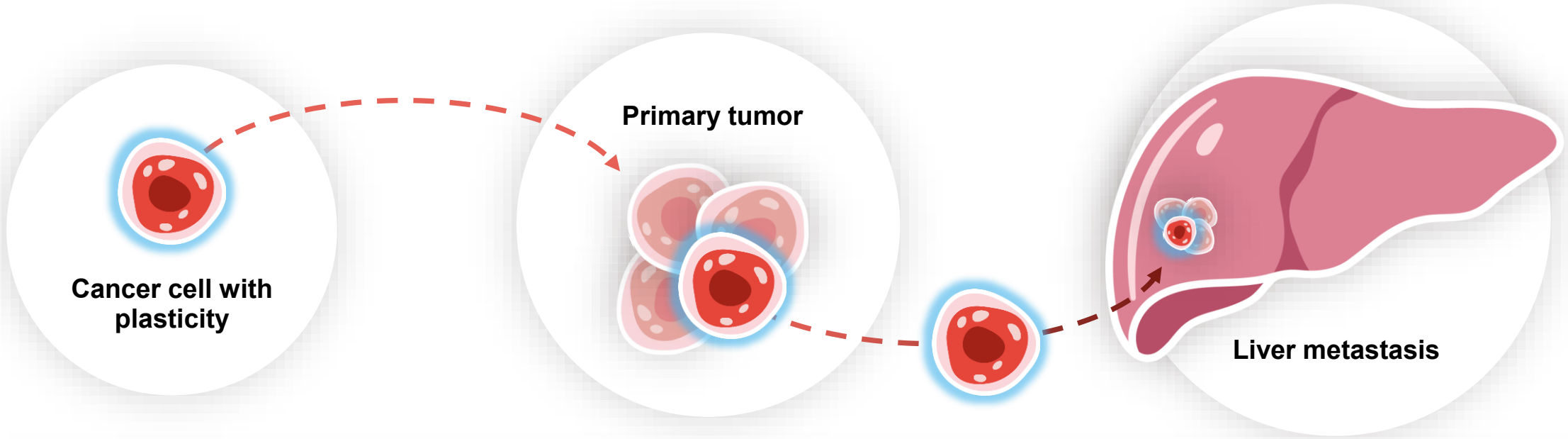


> Plasticity allows cancer cells to exist in a dynamic state with hybrid features of epithelial and mesenchymal cells[2]

1. Hanahan D. *Cancer Discov*. 2022;12(1):31-46; 2. Thompson EW, et al. *Nat Rev Clin Oncol.* 2025 Jul 22. doi: 10.1038/s41571-025-01058-2.

Cancer Cells With Plasticity Are a Small Sub-population That Can Initiate Tumors and Drive Metastasis[1–6]

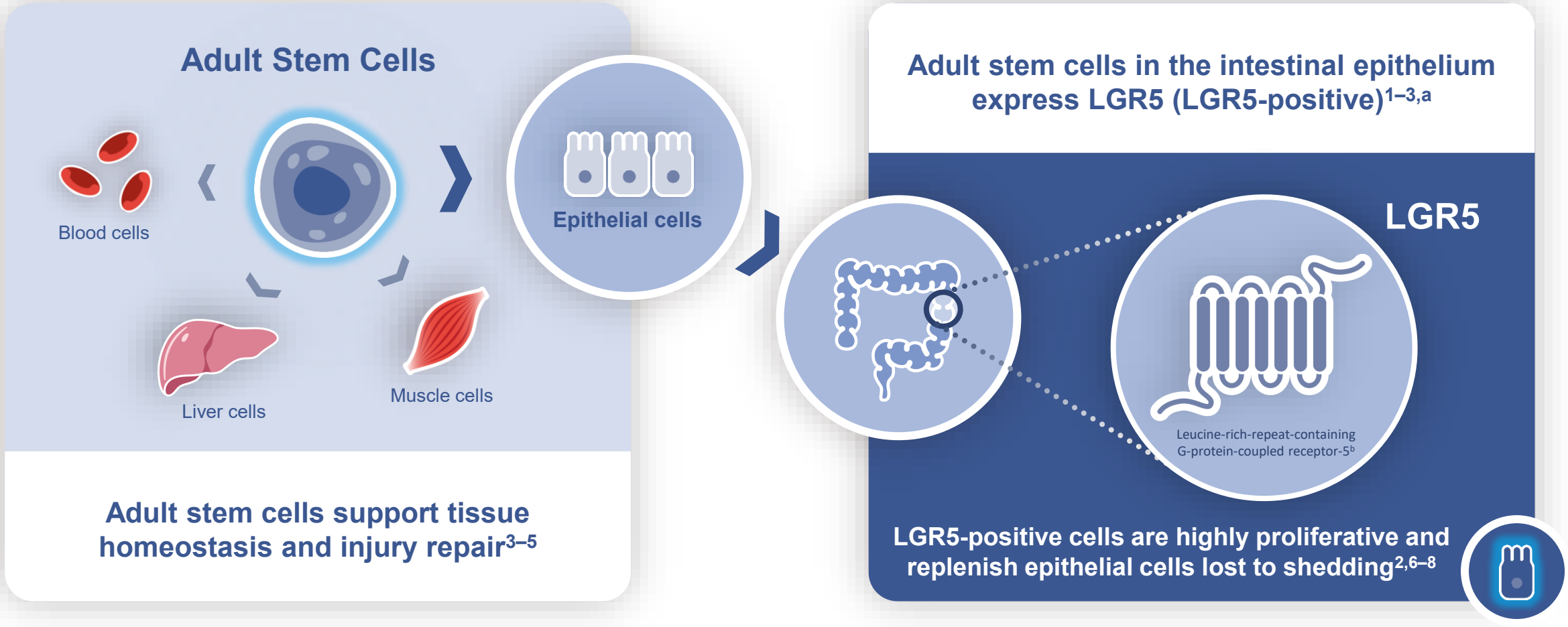
Plasticity = Stem-cell–like capabilities



Dynamic capabilities and extended longevity allow cancer cells with plasticity to be tumor-initiating[1,7,8]

1. Leung C et al. *Trends Cell Biol*. 2018;28(5):380-391; 2. Major AG et al. *Stem Cells Int*. 2013;2013:319489; 3. Kobayashi S et al. *Stem Cells.* 2012;30(12):2631-2644; 4. Quiroga M et al. *Cancers (Basel).* 2022;14(4):990; 5. Cao W et al. *Nat Commun*. 2020;11(1):1961; 6. Morgan RG et al. *Br J Cancer*. 2018;118(11):1410-1418; 7. Hirsch D et al. *Carcinogenesis*. 2014;35(4):849-858; 8. Barker N et al. *Nature*. 2009;457(7229).

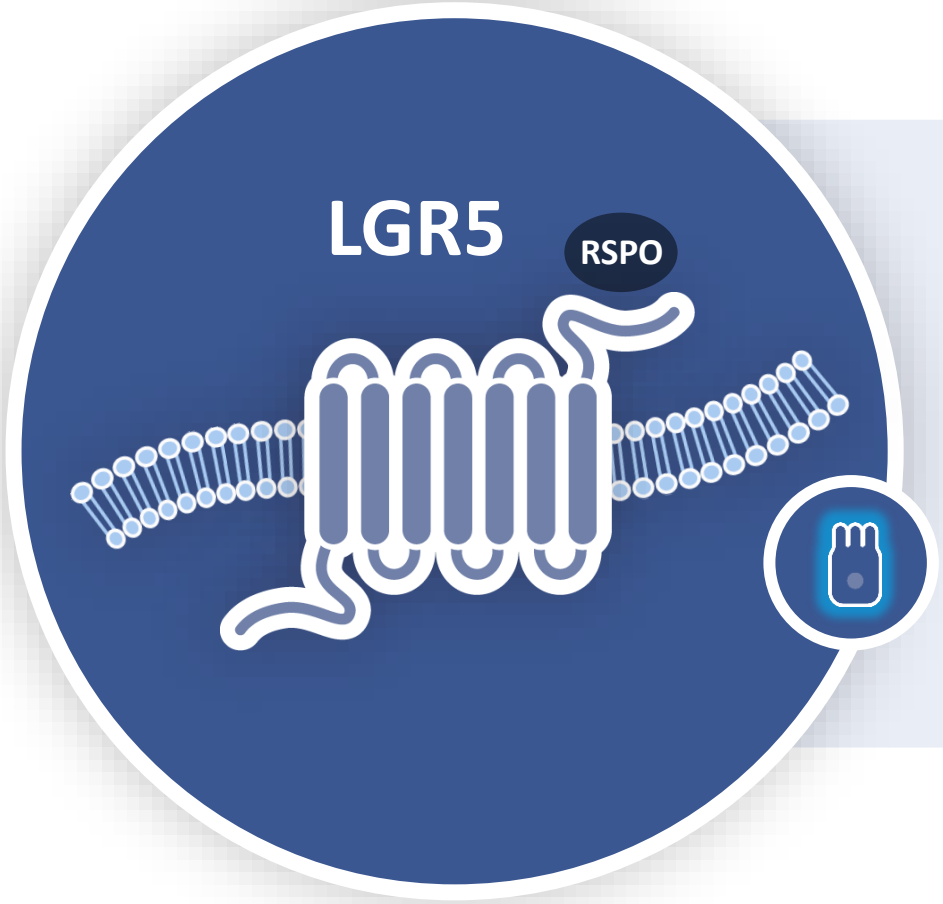
In Epithelial Tissue, Cells With Plasticity Express LGR5[1–3]



[a]Adult stem cells expressing LGR5 are also found in the mouth and lungs where they support tissue homeostasis[1]; [b]LGR5 is also known as GPR49.

1. Leung C et al. *Trends Cell Biol*. 2018;28(5):380-391; 2. de Lau W et al. *Genes Dev*. 2014;28(4):305-316; 3. Ge Y, Fuchs E. *Nat Rev Genet*. 201819:311-325; 4. Brunet A, et al. *Nat Rev Mol Cell Biol*. 2023;24:45-62; 5. National Institutes of Health. Stem cell basics. https://stemcells.nih.gov/info/basics/stc-basics. Accessed April 2025; 6. Barker N et al. *Cell Stem Cell*. 2010;6(1):25-36; 7. Basak O et al. *Cell Stem Cell*. 2017;20(2):177-190.e4; 8. Barker N et al. *Nature*. 2007;449(7165):1003-1007.

LGR5 Is an Epithelial Stem Cell Receptor Involved in Signaling and Proliferation[1,2]



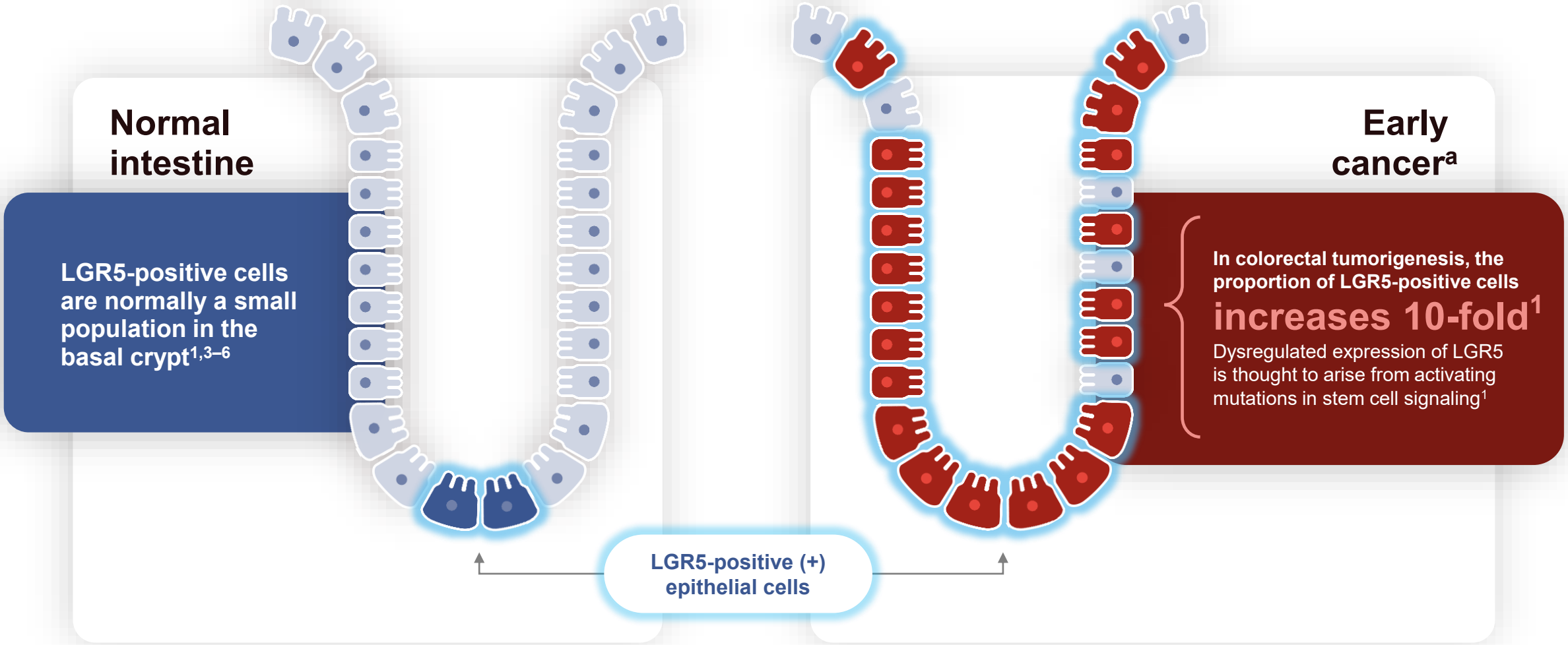
LGR5 is a receptor for R-Spondins (RSPOs)[1–4]

RSPOs are stem cell growth factors and potent signal enhancers[1–4]

LGR5-positive cells are self-renewing and long-lived (>6 months)[3,5–8]

1. de Lau W et al. *Genes Dev*. 2014;28(4):305-316; 2. Morgan RG et al. *Br J Cancer*. 2018;118(11):1410-1418; 3. Koo BK, Clevers H. *Gastroenterology*. 2014;147(2):289-302;
4. Carmon KS et al. *Mol Cell Biol*. 2012;32(11):2054-2064; 5. Barker N et al. *Cell Stem Cell*. 2010;6(1):25-36; 6. Basak O et al. *Cell Stem Cell*. 2017;20(2):177-190e4;
7. Barker N et al. *Nature*. 2007;449(7165):1003-1007; 8. Snippert HJ et al. *Cell*. 2010;143(1):134-144.

Cancer Cells That Express LGR5 Acquire Plasticity, Enabling Tumor Initiation[1,2]



[a]Adenomas and well/moderately differentiated adenocarcinomas.[1]

1. Martin ML, et al. *Cell Signal*. 2018;42:97-105; 2. Ge Y, Fuchs E. *Nat Rev Genet*. 201819:311-325; 3. de Lau W et al. *Genes Dev*. 2014;28(4):305-316; 4. Barker N et al. *Cell Stem Cell*. 2010;6(1):25-36; 5. Basak O et al. *Cell Stem Cell*. 2017;20(2):177-190 e4; 6. Barker N et al. *Nature*. 2007;449(7165):1003-1007.

Overexpression of LGR5 Occurs Across Carcinomas and Has Been Associated With Poor Outcomes[1–8]

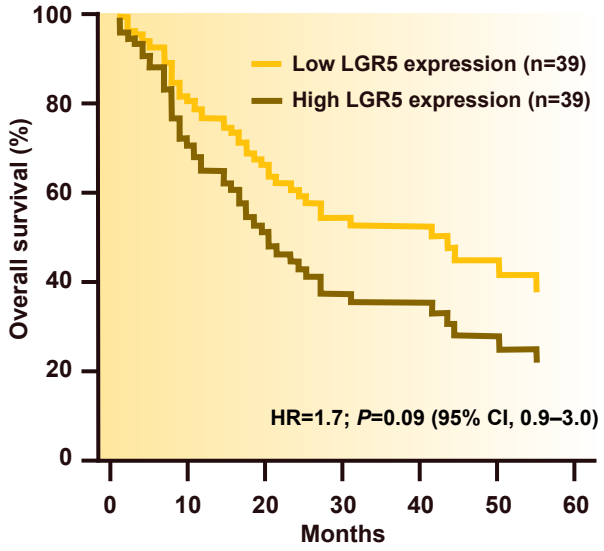
Colorectal cancer[4,5]

LGR5 overexpression was associated with:

- **30%** Reduction in disease-free survival (DFS, 1-year)[4,a]
- **3x** Increase in liver metastasis[4]
- **3x** Increase in disease recurrence (5-year)[5,b]

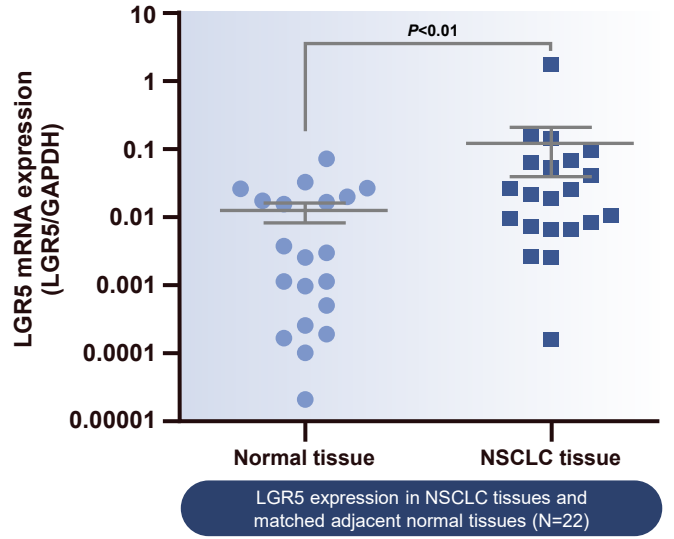
Head and neck cancer[6]

LGR5 overexpression was associated with poorer OS[c]



Lung cancer[7]

LGR5 was overexpressed in NSCLC tumor tissue



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Note: Each of the presented studies relies on mRNA expression for LGR5. There is no reliable commercial antibody for LGR5 detection. Studies evaluating LGR5 expression using immunohistochemistry (IHC) have produced conflicting results.[1,2,9]

[a]CRC patients who underwent radical tumor resection; [b]CRC stage II patients; [c]Patients with oral squamous cell carcinoma (OSCC). CI, confidence interval; HR, hazard ratio.

1. Leung C et al. *Trends Cell Biol*. 2018;28(5):380-391; 2. Morgan RG et al. *Br J Cancer*. 2018;118(11):1410-1418; 3. Xu L et al. *Stem Cell Res Ther*. 2019;10(1):219; 4. Takahashi H et al. *Ann Surg Oncol*. 2011;18:1166-74; 5. Stanisavljević L et al. *Acta Oncol*. 2016;55:1425-33; 6. Rot S et al. *BMC Cancer*. 2019;19(1):155; 7. Gao F et al. *Transl Cancer Res*. 2019;8(1):203–211; 8. Zhu Z et al. *PLoS One.* 2022;17(10):e0275679; 9. Mueller N et al. *Immunother Adv.* 2025;5(1):ltaf017.

LGR5 Enables the Activity of Cancer Cells by Accelerating Wnt/β-catenin Signaling[1–4,a]

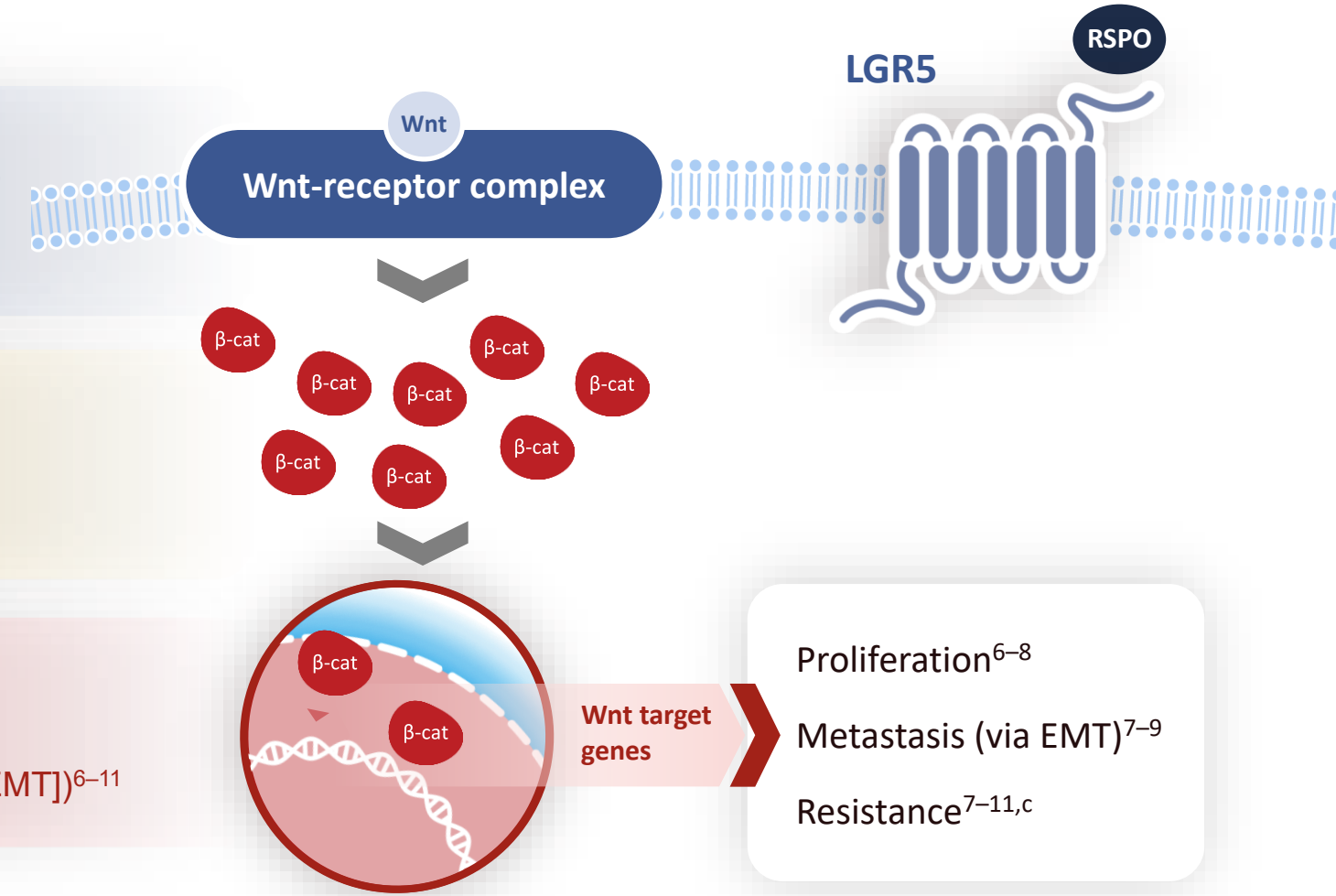
Wnt-signaling is a driver of tumorigenesis

β-catenin accumulates in the cell nucleus[5–7]

LGR5 increases levels of β-catenin[4,a]

β-catenin activates Wnt target genes[b]

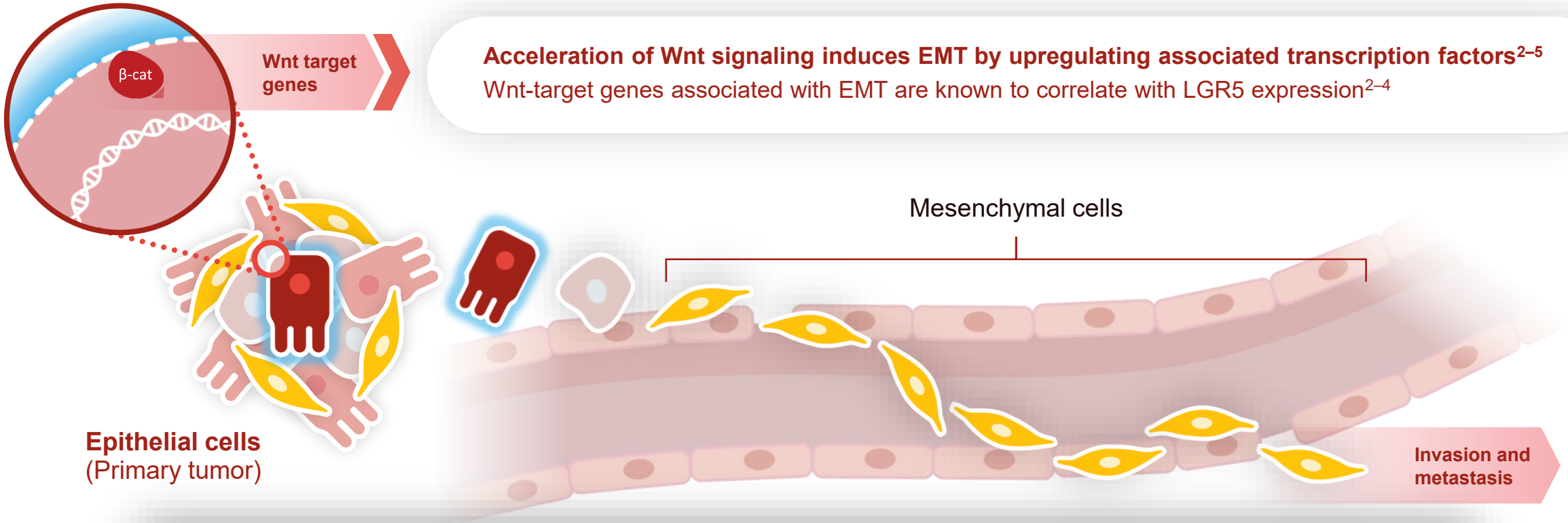
Drivers of proliferation, treatment resistance,[c] and metastasis (via epithelial-mesenchymal transition [EMT])[6–11]



[a]LGR5 is thought to accelerate Wnt/β-catenin signaling by sequestering enzymes (E3 ligases) that remove Wnt receptors from the cell surface.[12]
[b]LGR5 is a Wnt-target gene.[4]
[c]Resistance to chemotherapy, immune surveillance, and/or immune checkpoint inhibitors.

1. Leung C et al. *Trends Cell Biol*. 2018;28(5):380-391; 2. Hirsch D et al. *Carcinogenesis*. 2014;35(4):849-858; 3. Novellasdemunt L et al. *EMBO J*. 2020;39(3):e102771; 4. Carmon KS et al. *Mol Cell Biol*. 2012;32(11):2054-2064; 5. Major AG et al. *Stem Cells Int*. 2013;2013:319489; 6. de Lau W et al. *Genes Dev*. 2014;28(4):305-316; 7. Zhu Z et al. *PLoS One*. 2022;17(10):e0275679; 8. Liu J, et al. *Signal Transduct Target Ther*. 2022;7(1):3; 9. Patel S, et al. *Front Immunol*. 2019;10:2872; 10. Zhu G-X, et al. *Mol Med Rep*. 2021;23(2):105; 11. Liu X-S, et al. *Front Immunol*. 2019:10:1741; 12. Morgan RG et al. *Br J Cancer*. 2018;118(11):1410-1418.

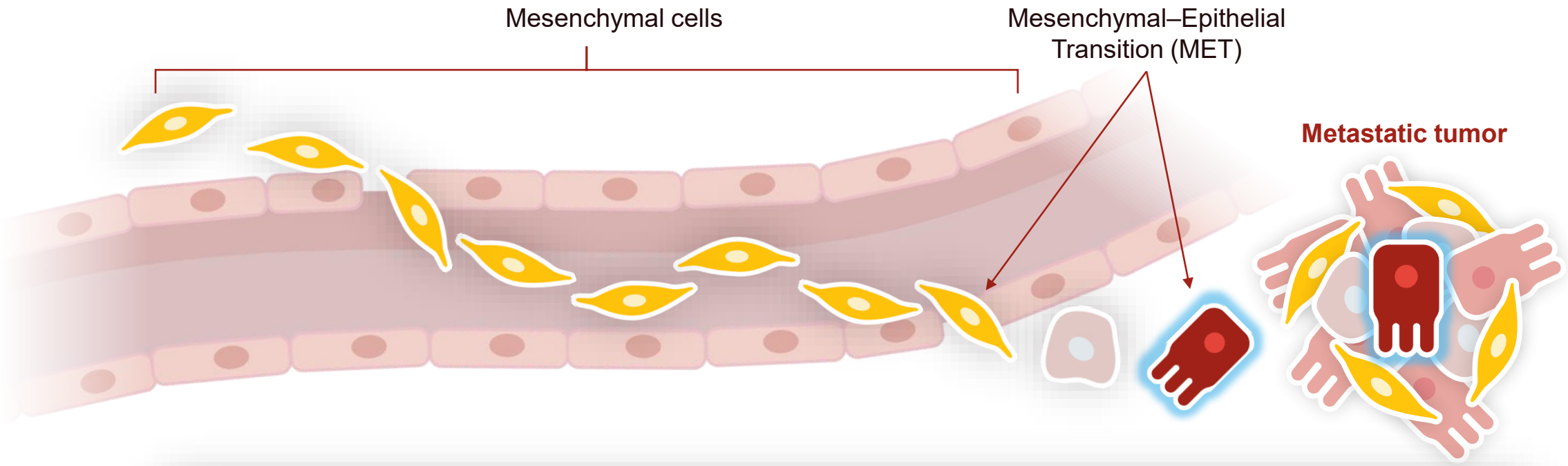
Wnt/β-catenin Signaling Is a Driver of EMT, Which Enables Cancer Cells to Leave the Primary Tumor Site and Metastasize in Other Organs[1–3]



1. Major AG et al. *Stem Cells Int*. 2013;2013:319489; 2. de Lau W et al. *Genes Dev*. 2014;28(4):305-316; 3. Rot S et al. *BMC Cancer*. 2019;19(1):155; 4. Li F et al. *Stem Cell Res*. 2020;47:101916; 5. Sun L et al. *Biomed Pharmacother*. 2024;175:116685; 6. Xu L et al. *Stem Cell Res Ther*. 2019;10(1):219; 7. de Sousa e Melo F et al. *Nature*. 2017;543(7647):676-680.

Once Cancer Cells Reach a New Environment, They Generate Metastatic Tumors by Restoring Epithelial Characteristics[1,2]

Pathogenic plasticity



The interplay between LGR5, Wnt/β-catenin signaling, and epithelial/mesenchymal transition enables tumor cells to invade surrounding tissue and initiate new tumors[3–6]

1. Xu Q et al. *Oncotarget*. 2017;8(6):9557-9571; 2. Yao D et al. *Mol Cancer Res*. 2011;9(12):1608-1620; 3. Li F et al. *Stem Cell Res*. 2020;47:101916; 4. Rot S et al. *BMC Cancer*. 2019;19(1):155; 5. Zhu Z et al. *PLoS One*. 2022;17(10):e0275679; 6. Conti S et a. *Nat Commun*. 2024;15(1):3363.

Pathogenic Plasticity

Key takeaways

1. **Plasticity drives epithelial cancer progression** by orchestrating the action of other hallmarks (eg, proliferation and dormancy, invasion and metastasis)[1]

2. Epithelial cancer **cells that express LGR5 acquire plasticity**, which enables tumor initiation, proliferation, and **metastasis**[2,3]

3. LGR5 contributes to pathogenic plasticity by accelerating **Wnt/β-catenin signaling and triggering EMT**[4–7]

1. Hanahan D. *Cancer Discov*. 2022;12(1):31-46; 2. Martin ML, et al. *Cell Signal*. 2018;42:97-105; 3. Ge Y, Fuchs E. *Nat Rev Genet*. 2018;19:311-325; 4. Leung C et al. *Trends Cell Biol*. 2018;28(5):380-391; 5. Hirsch D et al. *Carcinogenesis*. 2014;35(4):849-858; 6. Novellasdemunt L et al. *EMBO J*. 2020;39(3):e102771; 7. Carmon KS et al. *Mol Cell Biol*. 2012;32(11):2054-2064.

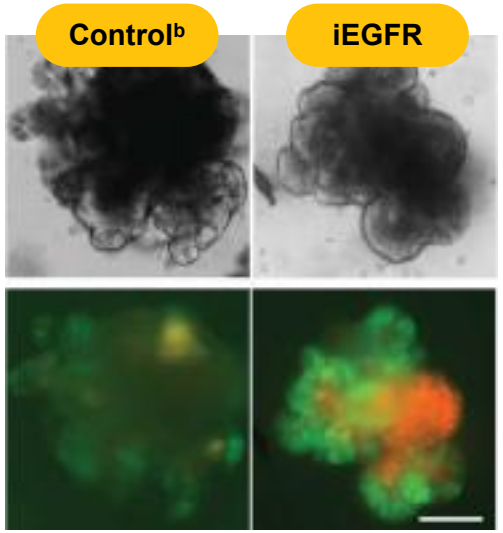
Chapter 3

LGR5, the Moving Target

Preclinical Data Show Increased Expression of LGR5 in Response to EGFR-targeted Therapies[1–4]

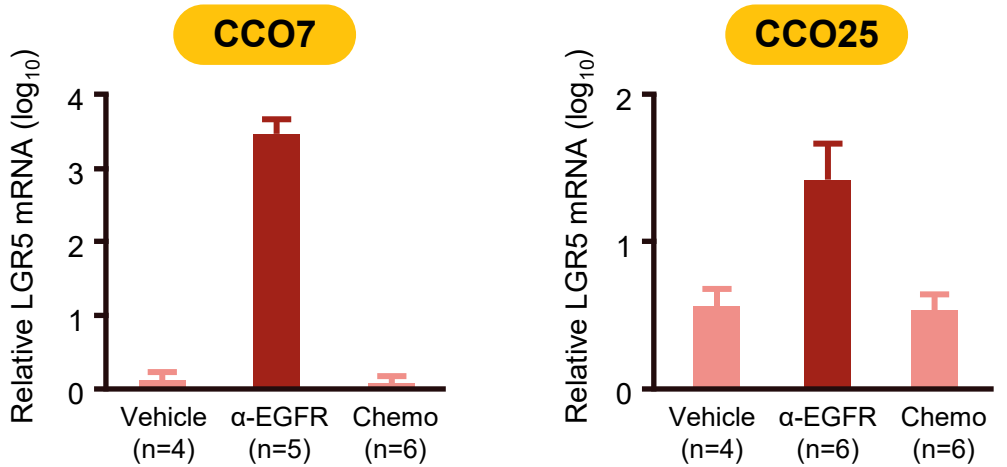
Pathogenic Plasticity in Action[a]

Increased expression of LGR5 after treatment with an EGFR RTK inhibitor (iEGFR)
(mouse intestinal organoid models)[2]



Reprinted from Basak O et al. Induced quiescence of Lgr5+ stem cells in intestinal organoids enables differentiation of hormone-producing enteroendocrine cells. *Cell Stem Cell*. 2017;20(2):177-190.e4, Copyright 2017, with permission from Elsevier.

Increased expression of LGR5 after treatment with an anti-EGFR monoclonal antibody
(human colon cancer organoids)[3]



Reprinted from Shimokawa M et al., Visualization and targeting of LGR5+ human colon cancer stem cells. Nature. 2017;545(7653):187-192, Copyright 2017, with permission from Springer Nature.

Is there a deadly dynamic between LGR5 and resistance to EGFR-targeted therapy?

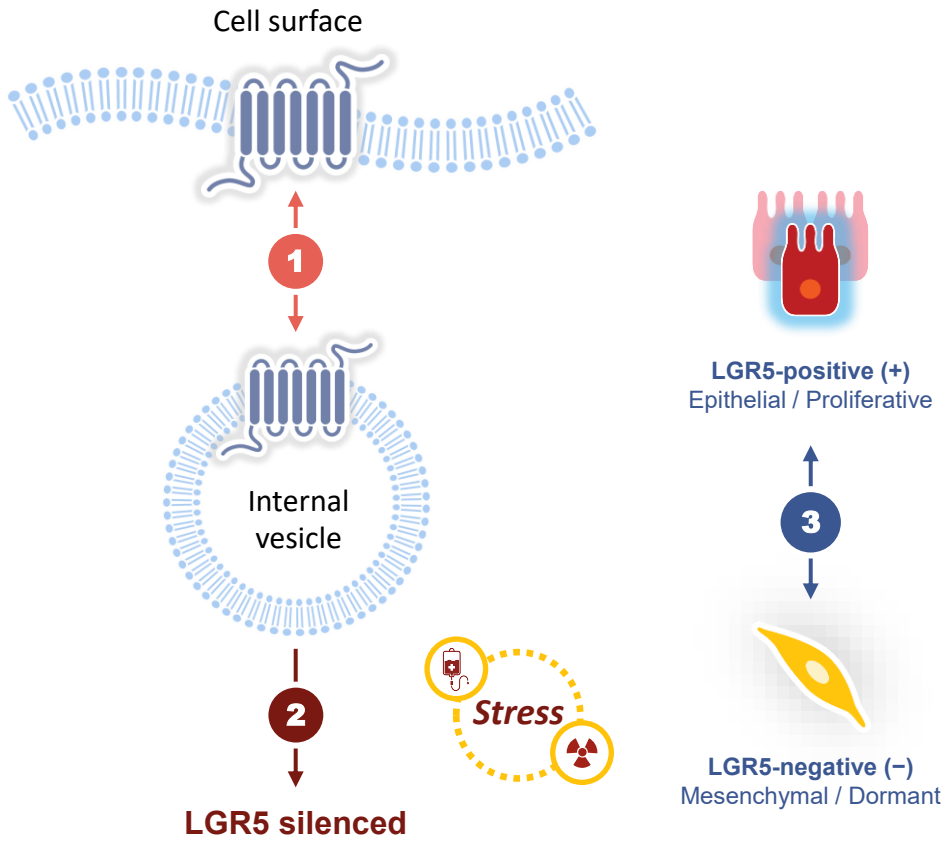
RTK, receptor tyrosine kinase.
[a]Cancers that undergo EMT are known to be resistant to EGFR inhibitors[5–8]; [b]Epidermal growth factor, Noggin, and R-spondin-1 (ENR).

1. High P et al. *Cell Rep Med.* 2025;0:102363; 2. Basak O et al. *Cell Stem Cell*. 2017;20(2):177-190.e4; 3. Shimokawa M et al. *Nature*. 2017;545(7653):187-192; 4. Lupo B et al. *Sci Transl Med*. 2020;12(555):eaax8313; 5. Schinke H et al. *Mol Cancer*. 2022;21(1):178; 6. Clement MS et al. *Transl Lung Cancer Res*. 2020;9(5):1904-1914; 7. Jakobsen KR et al. *Transl Lung Cancer Res*. 2016;5(2):172-182; 8. Kagohara LT et al. *Br J Cancer*. 2020;123(1):101-113.

LGR5 Is a Dynamic Regulator of Cancer Cell Plasticity[1–4]

Changes in Expression Drive Metastasis and Treatment Resistance

1. LGR5 is constantly internalized (even without RSPO) and replaced at the cell surface[5–8]
2. In response to negative stimuli (eg, ER stress,[a] nutrient deprivation[b]) LGR5 expression is silenced[6,7]
3. Transitioning between LGR5-positive and LGR5-negative states allows cancer cells to change phenotype and behavior[1–4]



[a]Cancer treatments (eg, cytotoxic drugs, radiation) can induce ER stress.[9] [b]Nutrient deprivation can be a driver of metastasis.[10]
ER, endoplasmic reticulum.

1. Fumagalli A et al. *Cell Stem Cell*. 2020;26(4):569-578.e7; 2. Walker F et al. *PLoS One.* 2011;6(7):e22733; 3. Fey SK et al. *Cell Rep.* 2024;43(6):114270; 4. Ge Y, Fuchs E. *Nat Rev Genet*. 2018;19:311-325;
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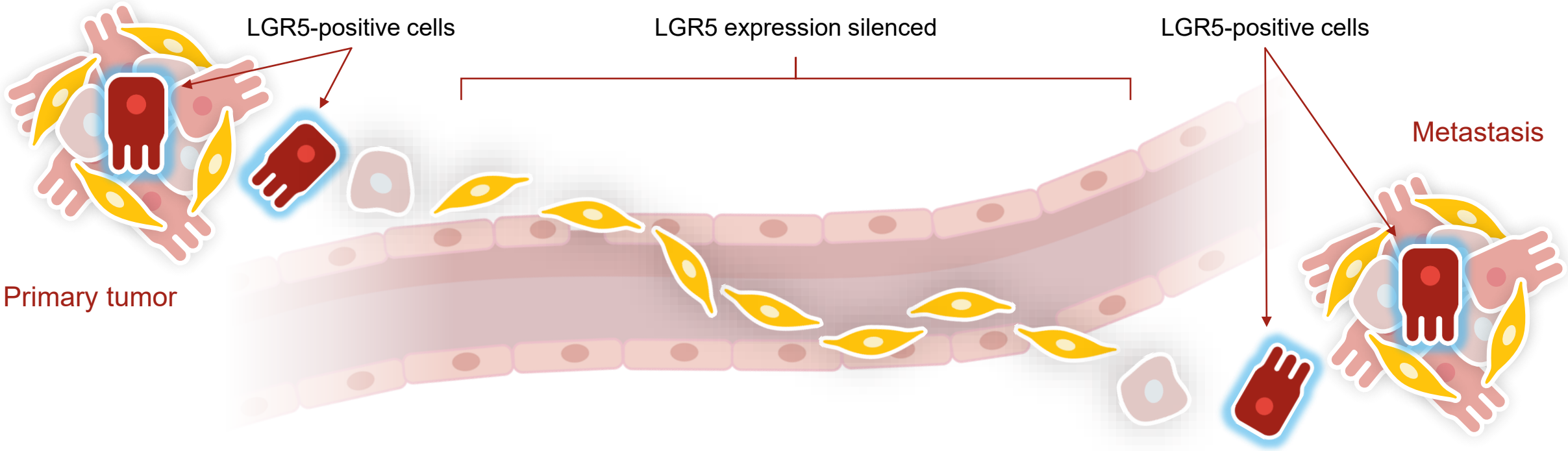
Reactivation of LGR5 Is Necessary for Metastatic Outgrowth[1,2]

LGR5 is a Moving Target



Developing an effective therapeutic strategy around LGR5 may require the integration of other targets[3–7]

1. Fumagalli A et al. *Cell Stem Cell*. 2020;26(4):569-578.e7; 2. Conti S et a. *Nat Commun*. 2024;15(1):3363; 3. Shimokawa M et al. *Nature*. 2017;545(7653):187-192;
4. Morgan RG et al. *Br J Cancer*. 2018;118(11):1410-1418; 5. Morgan RG et al. *Br J Cancer*. 2018;118(4):558-565; 6. Xu L et al. *Stem Cell Res Ther*. 2019;10(1):219;
7. Cao W et al. *Nat Commun*. 2020;11(1):1961.

LGR5, the Moving Target

Key takeaways

1. **EGFR-targeted therapy** has been shown to **increase** expression of **LGR5**[1–4,a]
2. Changes in **LGR5** expression allow cancer cells to **resist treatment and metastasize**[5–9]
3. Because **LGR5 is a dynamic protein**, developing an effective therapeutic strategy around it may require the **integration of other targets**[1,9–13]

[a]Based on preclinical data.

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Thank you!

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