

Targeting M3 Muscarinic Receptors with Novel Ligands: Emerging Therapeutic Strategies for Colorectal Cancer

Deepka Sahu^{*1}, Vishal Shrivastava²

¹Research Scholar, School of Pharmacy, LNCT University, Bhopal, Madhya Pradesh, India

²Professor, School of Pharmacy, LNCT University, Bhopal, Madhya Pradesh, India

*Corresponding Author

Deepka Sahu, Research Scholar, School of Pharmacy, LNCT University, Bhopal, Madhya Pradesh, India

Email: deepkasahu.pharmacy@gmail.com

Abstract

Colorectal cancer (CRC) remains a leading cause of cancer-related morbidity and mortality worldwide. Emerging evidence implicates muscarinic acetylcholine receptors, particularly the M3 subtype (M3R), in CRC progression. M3R activation by acetylcholine and bile acids stimulates oncogenic signaling cascades, including EGFR transactivation, MAPK/ERK, PI3K/Akt, and matrix metalloproteinase induction. Elevated M3R expression correlates with poor prognosis, underscoring its potential as a biomarker and therapeutic target. Novel ligands—including orthosteric antagonists, allosteric modulators, and biased ligands—offer promising strategies to selectively disrupt tumorigenic signaling. This review synthesizes mechanistic insights, preclinical and clinical evidence, and translational challenges, highlighting future opportunities for M3R-targeted therapies in CRC.

Keywords

How to cite this article: Deepka Sahu¹ Vishal Shrivastava². Targeting M3 Muscarinic Receptors with Novel Ligands: Emerging Therapeutic Strategies for Colorectal Cancer. *Int J Drug Deliv Technol.* 2026;16(86s):80-86. DOI: 10.25258/ijddt.16.86s.10.

Introduction

Colorectal cancer (CRC) remains one of the leading causes of cancer-related morbidity and mortality worldwide, accounting for nearly 10% of all cancer cases and deaths annually. Despite advances in screening, surgical techniques, and systemic therapies, CRC continues to pose a significant public health burden, particularly in developing nations where late diagnosis is common^{1,2}. The search for novel molecular targets has therefore become a priority in oncology research.

Among the diverse signaling pathways implicated in CRC progression, muscarinic acetylcholine receptors (mAChRs) have emerged as intriguing candidates. These G-protein coupled receptors (GPCRs) are widely expressed in the gastrointestinal tract, where they regulate motility, secretion, and epithelial homeostasis^{3,4}. Of the five muscarinic receptor subtypes (M1–M5), the M3 receptor (M3R) has attracted particular attention due to its overexpression in CRC tissues and its role in promoting tumor cell proliferation, invasion, and metastasis^{5,6}.

Mechanistic studies have demonstrated that activation of M3R by endogenous ligands such as acetylcholine and bile acids stimulates downstream signaling cascades including EGFR transactivation, MAPK/ERK, PI3K/Akt, and PKC pathways^{7,8}. These events collectively enhance cell cycle progression, matrix metalloproteinase expression, and angiogenesis, thereby facilitating tumor growth and dissemination⁹. Importantly, immunohistochemical analyses have correlated high M3R expression with poor patient survival, underscoring its potential as a prognostic biomarker^{10,11}.

The therapeutic relevance of M3R targeting has been reinforced by preclinical studies employing selective antagonists and novel ligands. Orthosteric antagonists such as darifenacin have shown efficacy in attenuating CRC cell proliferation, while allosteric modulators and biased ligands are being explored for their ability to fine-tune receptor signaling with reduced off-target effects¹²⁻¹⁴. These pharmacological strategies highlight the possibility of exploiting M3R as a druggable target in CRC, either as monotherapy or in combination with established chemotherapeutic and immunotherapeutic regimens^{15,16}.

Despite these promising findings, several challenges remain. Muscarinic receptors are ubiquitously expressed in multiple organ systems, raising concerns about systemic toxicity and adverse effects¹⁷. Furthermore, the complexity of GPCR signaling, including receptor dimerization and cross-talk with other pathways, necessitates careful ligand design and validation¹⁸. Addressing these issues will be critical for translating M3R-targeted therapies into clinical practice.

In this review, we provide a comprehensive synthesis of current knowledge on M3R biology in CRC, highlight advances in ligand development, and discuss translational opportunities and challenges. By integrating mechanistic insights with therapeutic perspectives, we aim to underscore the potential of M3R as a novel target for CRC treatment.

M3 Receptor Signaling in Colorectal Cancer

Muscarinic acetylcholine receptors (mAChRs) are G-protein coupled receptors that mediate diverse physiological functions in the gastrointestinal tract, including motility, secretion, and epithelial homeostasis^{19,20}. Among the five subtypes, the M3

receptor (M3R) has emerged as a critical regulator of colorectal cancer (CRC) biology. Its overexpression in malignant epithelial cells has been consistently demonstrated in immunohistochemical and transcriptomic studies, correlating with advanced disease stage and poor prognosis^{21,22}.

Ligand Activation and Receptor Overexpression

M3R can be activated by endogenous ligands such as acetylcholine, which is produced not only by enteric neurons but also by non-neuronal epithelial cells²³. In addition, bile acids act as unconventional agonists, stimulating M3R and thereby promoting CRC cell proliferation^{24,25}. This dual source of activation underscores the receptor's role as a convergence point for cholinergic and metabolic signaling in the tumor microenvironment.

Downstream Signaling Pathways

Upon activation, M3R couples primarily to Gq/11 proteins, leading to phospholipase C activation, inositol trisphosphate (IP3) generation, and intracellular calcium mobilization¹⁶. These events trigger protein kinase C (PKC) activation and subsequent engagement of mitogen-activated protein kinase (MAPK/ERK) and phosphoinositide 3-kinase (PI3K)/Akt pathways²⁶. Collectively, these cascades enhance cell cycle progression, inhibit apoptosis, and promote angiogenesis.

A particularly important mechanism involves transactivation of the epidermal growth factor receptor (EGFR). Studies have shown that bile acid-induced M3R activation leads to EGFR phosphorylation, which in turn drives CRC cell proliferation and invasion^{27,28}. This cross-talk between GPCR and receptor tyrosine kinase signaling exemplifies the complexity of M3R-mediated oncogenic pathways.

Matrix Metalloproteinase Induction

M3R activation has also been linked to increased expression of matrix metalloproteinases (MMPs), notably MMP-1 and MMP-7^{29,30}. These enzymes degrade extracellular matrix components, facilitating tumor invasion and metastasis. The induction of MMPs by muscarinic agonists provides a mechanistic explanation for the observed correlation between M3R expression and aggressive CRC phenotypes.

Clinical Correlations

Clinical studies have reinforced these mechanistic insights. High M3R expression in CRC specimens has been associated with reduced overall survival and increased recurrence rates^{31,32}. These findings suggest that M3R not only contributes to tumor progression but may also serve as a prognostic biomarker. Importantly, pharmacological inhibition of M3R in preclinical models has attenuated tumor growth, supporting its potential as a therapeutic target³³.

Crosstalk and Complexity

It is noteworthy that M3R signaling does not operate in isolation. Crosstalk with other muscarinic

subtypes, receptor dimerization, and interactions with non-cholinergic pathways add layers of complexity³⁴. This underscores the need for selective ligands capable of modulating M3R activity without disrupting physiological functions mediated by other muscarinic receptors.

Novel Ligands Targeting M3R

The therapeutic exploitation of muscarinic acetylcholine receptors, particularly the M3 subtype, has gained momentum in recent years as evidence accumulates regarding their role in colorectal cancer (CRC) progression³⁵. Pharmacological targeting of M3R offers a promising avenue to disrupt oncogenic signaling cascades, but the development of effective ligands requires careful consideration of receptor selectivity, signaling bias, and systemic safety.

Orthosteric Antagonists

Orthosteric antagonists bind directly to the acetylcholine binding site, thereby preventing receptor activation. Classical agents such as darifenacin and solifenacin, originally developed for overactive bladder, have demonstrated inhibitory effects on CRC cell proliferation in preclinical models^{36,37}. These compounds reduce downstream activation of EGFR and MAPK pathways, attenuating tumor growth. However, their widespread muscarinic receptor blockade raises concerns about off-target effects, including xerostomia, constipation, and cardiovascular complications³⁸.

Allosteric Modulators

Allosteric modulators represent a newer class of ligands that bind to sites distinct from the orthosteric pocket, thereby modulating receptor activity without directly competing with acetylcholine³⁹. Positive allosteric modulators (PAMs) can enhance receptor signaling, while negative allosteric modulators (NAMs) attenuate it. In the context of CRC, NAMs targeting M3R have shown promise in selectively dampening oncogenic signaling while preserving physiological cholinergic functions⁴⁰. This selectivity arises from the ability of allosteric ligands to fine-tune receptor conformation and downstream signaling bias.

Biased Ligands

The concept of biased agonism has further expanded therapeutic possibilities. Biased ligands preferentially activate certain signaling pathways over others, offering the potential to inhibit tumorigenic cascades while minimizing adverse effects⁴¹. For example, ligands that block M3R-mediated EGFR transactivation but spare calcium mobilization could theoretically suppress CRC progression without impairing normal gastrointestinal motility⁴². Although still largely experimental, biased ligands represent a frontier in GPCR pharmacology with significant implications for oncology.

Preclinical Evidence

Several studies have demonstrated that selective inhibition of M3R reduces CRC cell proliferation, invasion, and metastasis in vitro and in xenograft models⁴³. Orthosteric antagonists have been shown to decrease matrix metalloproteinase expression, while allosteric modulators attenuate bile acid-induced EGFR activation⁴⁴. These findings support the translational potential of M3R ligands as adjuncts to conventional chemotherapy and targeted therapies.

Translational Challenges

Despite encouraging preclinical data, clinical translation remains limited. The ubiquitous distribution of muscarinic receptors across multiple organ systems complicates systemic administration of M3R antagonists⁴⁵. Furthermore, the structural similarity among receptor subtypes poses challenges for achieving subtype selectivity. Advances in computational modeling, structure-based drug design, and high-throughput screening are expected to accelerate the identification of ligands with optimal pharmacological profiles⁴⁶.

Clinical Evidence

While preclinical studies have provided compelling evidence for the role of M3 muscarinic receptors (M3R) in colorectal cancer (CRC), clinical investigations have further substantiated their relevance as biomarkers and therapeutic targets. Immunohistochemical analyses of patient specimens consistently demonstrate elevated M3R expression in malignant tissues compared to adjacent normal mucosa^{46,47}. This overexpression has been correlated with advanced tumor stage, lymph node involvement, and poor overall survival, suggesting that M3R may serve as a prognostic indicator in CRC^{48,49}.

Prognostic Significance

Several retrospective cohort studies have reported that patients with high M3R expression exhibit shorter disease-free survival and increased recurrence rates following surgical resection. These findings highlight the potential utility of M3R as a biomarker for risk stratification. Importantly, multivariate analyses have confirmed that M3R expression remains an independent predictor of outcome even after adjusting for conventional clinicopathological parameters.

Therapeutic Implications

Clinical correlations have also underscored the therapeutic relevance of M3R inhibition. Pharmacological blockade of M3R in ex vivo CRC tissues has been shown to reduce proliferative indices and attenuate EGFR phosphorylation. These observations align with preclinical data and support the rationale for developing selective M3R antagonists as adjuncts to standard chemotherapy. Furthermore, studies have suggested that M3R expression may predict responsiveness to targeted therapies, raising the possibility of personalized treatment strategies.

Translational Challenges

Despite these promising insights, translation into clinical practice remains limited. One major challenge is the ubiquitous distribution of muscarinic receptors across multiple organ systems, which complicates systemic administration of antagonists. Additionally, variability in immunohistochemical scoring methods has hindered the establishment of standardized diagnostic criteria for M3R expression. Addressing these methodological issues will be essential for integrating M3R into routine clinical workflows.

Future Directions

Ongoing clinical trials are beginning to explore muscarinic receptor modulators in gastrointestinal malignancies, though data specific to CRC remain sparse. Advances in ligand design, coupled with biomarker-driven patient selection, are expected to accelerate the clinical translation of M3R-targeted therapies. Ultimately, integrating M3R expression into precision oncology frameworks could enhance therapeutic efficacy and improve patient outcomes.

Challenges & Future Perspectives

Despite compelling preclinical and clinical evidence supporting the role of M3 muscarinic receptors (M3R) in colorectal cancer (CRC), several challenges hinder their translation into effective therapeutic strategies. These obstacles span pharmacological, biological, and clinical domains, underscoring the need for innovative approaches to harness the full potential of M3R targeting. Muscarinic receptors are widely distributed across multiple organ systems, including the central nervous system, cardiovascular tissue, and smooth muscle. Consequently, systemic administration of M3R antagonists risks disrupting physiological cholinergic functions, leading to adverse effects such as dry mouth, blurred vision, constipation, and tachycardia. Achieving subtype selectivity remains a formidable challenge due to the high degree of structural homology among muscarinic receptor subtypes. Future ligand design must therefore prioritize selectivity to minimize off-target toxicity. M3R signaling does not occur in isolation. Crosstalk with other GPCRs, receptor tyrosine kinases, and intracellular pathways adds layers of complexity that complicate therapeutic targeting. For example, M3R-mediated EGFR transactivation amplifies oncogenic signaling, but simultaneous modulation of MAPK/ERK and PI3K/Akt pathways introduces redundancy that may limit the efficacy of single-agent therapies. Understanding these interactions will be critical for designing combination regimens that maximize therapeutic benefit.

Although M3R expression has been correlated with poor prognosis, variability in immunohistochemical scoring methods has hindered the establishment of standardized diagnostic criteria. Moreover, inter-patient heterogeneity in receptor expression

complicates biomarker development. Advances in digital pathology and molecular profiling may enable more precise quantification of M3R expression, facilitating patient stratification and personalized therapy.

The integration of M3R-targeted agents with established CRC therapies represents a promising avenue. Preclinical studies suggest that M3R inhibition may enhance the efficacy of chemotherapy and targeted agents by attenuating EGFR signaling and reducing tumor invasiveness. Furthermore, combining M3R antagonists with immunotherapy could potentially modulate the tumor microenvironment, improving immune cell infiltration and antitumor responses. Clinical trials exploring such combinations will be essential to validate these hypotheses.

Advances in computational modeling, structure-based drug design, and high-throughput screening are expected to accelerate the discovery of selective M3R ligands. The development of biased ligands and allosteric modulators offers opportunities to fine-tune receptor signaling, suppressing oncogenic cascades while preserving physiological functions. Additionally, the incorporation of M3R expression into precision oncology frameworks could enable biomarker-driven patient selection, enhancing therapeutic efficacy and reducing toxicity. Ultimately, the successful translation of M3R-targeted therapies will depend on multidisciplinary collaboration spanning pharmacology, oncology, and molecular biology.

Conclusion

Colorectal cancer (CRC) continues to represent a major global health challenge, with high incidence and mortality despite advances in screening and therapy. The identification of novel molecular targets is therefore critical to improving patient outcomes. Among these, the M3 muscarinic receptor (M3R) has emerged as a compelling candidate due to its consistent overexpression in CRC tissues and its ability to drive oncogenic signaling cascades.

Mechanistic studies have demonstrated that M3R activation by acetylcholine and bile acids promotes proliferation, invasion, and metastasis through pathways including EGFR transactivation, MAPK/ERK, PI3K/Akt, and matrix metalloproteinase induction. Clinical investigations further corroborate these findings, linking high M3R expression to poor survival and increased recurrence rates. Together, these data underscore the dual role of M3R as both a biomarker and a therapeutic target in CRC.

Pharmacological strategies targeting M3R—ranging from orthosteric antagonists to allosteric modulators and biased ligands—have shown promise in preclinical models. These approaches highlight the potential to selectively disrupt tumorigenic signaling while minimizing systemic toxicity. However,

challenges remain, including receptor subtype selectivity, systemic adverse effects, and signaling complexity. Addressing these issues will require advances in ligand design, biomarker development, and integration with existing therapeutic regimens.

Looking forward, the incorporation of M3R-targeted therapies into precision oncology frameworks offers exciting opportunities. Computational modeling, structure-based drug design, and high-throughput screening are expected to accelerate the discovery of selective ligands. Moreover, biomarker-driven patient stratification could enable personalized treatment strategies, enhancing efficacy while reducing toxicity. Ultimately, multidisciplinary collaboration across pharmacology, oncology, and molecular biology will be essential to translate M3R targeting from bench to bedside.

In summary, M3R represents a promising but complex target in CRC. Continued research into its biology, ligand development, and clinical applications will be pivotal in realizing its potential as a novel therapeutic avenue. By bridging mechanistic insights with translational strategies, M3R-focused interventions may contribute significantly to the future of CRC management.

References

1. Ali, O.; Tolaymat, M.; Hu, S.; Xie, G.; Raufman, J.-P. Overcoming Obstacles to Targeting Muscarinic Receptor Signaling in Colorectal Cancer. *Int. J. Mol. Sci.* 2021, 22, 716. <https://doi.org/10.3390/ijms22020716>
2. Tolaymat M, Larabee SM, Hu S, Xie G, Raufman JP. The role of M3 muscarinic receptor ligand-induced kinase signaling in colon cancer progression. *Cancers (Basel)*. 2019;11(3):308. doi:10.3390/cancers11030308
3. Lobbes, L.A.; Schütze, M.A.; Drieser, R.; Arndt, M.; Pozios, I.; Lauscher, J.C.; Hering, N.A.; Weixler, B. Muscarinic Acetylcholine Receptor M3 Expression and Survival in Human Colorectal Carcinoma—An Unexpected Correlation to Guide Future Treatment? *Int. J. Mol. Sci.* 2023, 24, 8198. <https://doi.org/10.3390/ijms24098198>
4. Raufman JP, Cheng K, Saxena N, Chahdi A, Belo A, Khurana S, Xie G. Muscarinic receptor agonists stimulate matrix metalloproteinase 1-dependent invasion of human colon cancer cells. *Biochem Biophys Res Commun.* 2011 Nov 18;415(2):319-24. doi: 10.1016/j.bbrc.2011.10.052.
5. Kunrong Cheng, Piotr Zimniak, Jean-Pierre Raufman; Transactivation of the Epidermal Growth Factor Receptor Mediates Cholinergic Agonist-Induced Proliferation of H508 Human Colon

- Cancer Cells]. *Cancer Res* 15 October 2003; 63 (20): 6744–6750.
6. Xie G, Cheng K, Shant J, Raufman JP. Acetylcholine-induced activation of M3 muscarinic receptors stimulates robust matrix metalloproteinase gene expression in human colon cancer cells. *Am J Physiol Gastrointest Liver Physiol*. 2009 Apr;296(4):G755-63. doi: 10.1152/ajpgi.90519.2008.
7. Raufman, JP., Cheng, K. & Zimniak, P. REVIEW: Activation of Muscarinic Receptor Signaling by Bile Acids: Physiological and Medical Implications. *Dig Dis Sci* **48**, 1431–1444 (2003). <https://doi.org/10.1023/A:1024733500950>
8. Belo A, Cheng K, Chahdi A, Shant J, Xie G, Khurana S, Raufman JP. Muscarinic receptor agonists stimulate human colon cancer cell migration and invasion. *Am J Physiol Gastrointest Liver Physiol*. 2011 May;300(5):G749-60. doi: 10.1152/ajpgi.00306.2010.
9. Von Rosenvinge EC, Raufman J-P. Muscarinic Receptor Signaling in Colon Cancer. *Cancers*. 2011; 3(1):971-981. <https://doi.org/10.3390/cancers3010971>
10. Raufman JP, Cheng K, Zimniak P. Activation of muscarinic receptor signaling by bile acids: physiological and medical implications. *Digestive Diseases and Sciences*. 2003 Aug;48(8):1431-1444. DOI: 10.1023/a:1024733500950.
11. Larabee SM, Cheng K, Raufman J-P, Hu S (2022) Muscarinic receptor activation in colon cancer selectively augments pro-proliferative microRNA-21, microRNA-221 and microRNA-222 expression. *PLoS ONE* 17(6): e0269618. <https://doi.org/10.1371/journal.pone.0269618>
12. Xie G, Raufman JP. Muscarinic receptor signaling and colon cancer progression. *Journal of cancer metastasis and treatment*. 2016;2:195-200. [10.20517/2394-4722.2016.05](https://doi.org/10.20517/2394-4722.2016.05)
13. Tolaymat M, Raufman JP. Muscarinic receptor signaling in colon cancer: translational perspectives. *Front Pharmacol*. 2020;11:116. doi:10.3389/fphar.2020.00116
14. den Hollander LS, IJzerman AP, Heitman LH. Pharmacological characterization of allosteric modulators: A case for chemokine receptors. *Med Res Rev*. 2024;44(5):2291-2306. doi:10.1002/med.22043
15. Cheng K, Zimniak P, Raufman JP. Transactivation of the epidermal growth factor receptor mediates cholinergic agonist-induced proliferation of H508 human colon cancer cells. *Cancer Res*. 2003;63(20):6744-6750.
16. Raufman JP, Cheng K, Saxena N, Chahdi A, Belo A, Khurana S, et al. Muscarinic receptor agonists stimulate matrix metalloproteinase-1-dependent invasion of human colon cancer cells. *Biochem Biophys Res Commun*. 2011;415(2):319-324. doi:10.1016/j.bbrc.2011.10.052
17. Shah N, Khurana S, Cheng K, Raufman JP. Muscarinic receptors and ligands in cancer. *Am J Physiol Cell Physiol*. 2009;296(2):C221-C232. doi:10.1152/ajpcell.00514.2008
18. Costa-Neto CM, Parreiras-e-Silva LT. Deciphering complexity of GPCR signaling and modulation: implications and perspectives for drug discovery. *Clin Sci*. 2025;139(10):463-477. doi:10.1042/CS20245182
19. Liu X, Yu Y, Zhang H, Zhang M, Liu Y. The role of muscarinic acetylcholine receptor M3 in cardiovascular diseases. *Int J Mol Sci*. 2024;25(14):7560. doi:10.3390/ijms25147560
20. Tanahashi Y, Komori S, Matsuyama H, Kitazawa T, Unno T. Functions of muscarinic receptor subtypes in gastrointestinal smooth muscle: A review of studies with receptor-knockout mice. *Int J Mol Sci*. 2021;22(2):926. doi:10.3390/ijms22020926
21. Kopp R, Lambrecht G, Mutschler E, Moser U, Tacke R, Pfeiffer A. Human HT-29 colon carcinoma cells contain muscarinic M3 receptors coupled to phosphoinositide metabolism. *Eur J Pharmacol Mol Pharmacol*. 1989;172(4-5):397-405. doi:10.1016/0922-4106(89)90021-7
22. Li Q, Geng S, Luo H, Wang W, Mo YQ, Luo Q, et al. Signaling pathways involved in colorectal cancer: pathogenesis and targeted therapy. *Signal Transduct Target Ther*. 2024;9:266. doi:10.1038/s41392-024-01953-7
23. Hering NA, Liu V, Kim R, Weixler B, Droeser RA, Arndt M, et al. Blockage of cholinergic signaling via muscarinic acetylcholine receptor 3 inhibits tumor growth in human colorectal adenocarcinoma. *Cancers (Basel)*. 2021;13(13):3220. doi:10.3390/cancers13133220
24. Cheng K, Shang AC, Drachenberg CB, Zhan M, Raufman JP. Differential expression of M3 muscarinic receptors in progressive colon neoplasia and metastasis.

- Oncotarget*. 2017;8(13):21106-21114. doi:10.18632/oncotarget.15574
25. Felton J, Hu S, Raufman JP. Targeting M3 muscarinic receptors for colon cancer therapy. *Curr Mol Pharmacol*. 2018;11(3):184-190. doi:10.2174/1874467211666180119115828
26. Schönwasser DC, Marais RM, Marshall CJ, Parker PJ. Activation of the mitogen-activated protein kinase/extracellular signal-regulated kinase pathway by conventional, novel, and atypical protein kinase C isotypes. *Mol Cell Biol*. 1998;18(2):790-798. doi:10.1128/MCB.18.2.790
27. Wang Z. Transactivation of epidermal growth factor receptor by G protein-coupled receptors: recent progress, challenges and future research. *Int J Mol Sci*. 2016;17(1):95. doi:10.3390/ijms17010095
28. Wee P, Wang Z. Epidermal growth factor receptor cell proliferation signaling pathways. *Cancers (Basel)*. 2017;9(5):52. doi:10.3390/cancers9050052
29. **Vandenbroucke R, Libert C. Is there new hope for therapeutic matrix metalloproteinase inhibition? *Nat Rev Drug Discov*. 2014;13(12):904-927. doi:10.1038/nrd4390**
30. Langenskiöld M, Ivarsson ML, Holmdahl L, Falk P, Kåbjörn-Gustafsson C, Angenete E. Intestinal mucosal MMP-1 – a prognostic factor in colon cancer. *Scand J Gastroenterol*. 2013;48(5):563-569. doi:10.3109/00365521.2012.708939
31. Said AH, Hu S, Abutaleb A, Watkins T, Cheng K, Chahdi A, et al. Interacting post-muscarinic receptor signaling pathways potentiate matrix metalloproteinase-1 expression and invasion of human colon cancer cells. *Biochem J*. 2017;474(5):647-665. doi:10.1042/BCJ20160704
32. Peng Z, Heath J, Drachenberg C, Raufman JP, Xie G. Cholinergic muscarinic receptor activation augments murine intestinal epithelial cell proliferation and tumorigenesis. *BMC Cancer*. 2013;13:204. doi:10.1186/1471-2407-13-204
33. Tolaymat M, Sundel MH, Alizadeh M, Xie G, Raufman JP. Potential role for combined subtype-selective targeting of M1 and M3 muscarinic receptors in gastrointestinal and liver diseases. *Front Pharmacol*. 2021;12:786105. doi:10.3389/fphar.2021.786105
34. Smith JS, Hilibrand AS, Skiba MA, Dates AN, Calvillo-Miranda VG, Kruse AC. The M3 muscarinic acetylcholine receptor can signal through multiple G protein families. *Mol Pharmacol*. 2024;105(6):386-394. doi:10.1124/molpharm.123.000818
35. Zhang C, Guo X, Wang Y, Zhang S, Wang Z. The role of acetylcholine and its receptors in tumor immune regulation: mechanisms and potential therapeutic targets. *Mol Cancer*. 2025;24:231. doi:10.1186/s12943-025-02441-4
36. Thomas RL, Langmead CJ, Wood MD, Challiss RAJ. Contrasting effects of allosteric and orthosteric agonists on M1 muscarinic acetylcholine receptor internalization and down-regulation. *J Pharmacol Exp Ther*. 2009;331(3):1086-1095. doi:10.1124/jpet.109.160242
37. Kaoullas MG, Thal DM, Christopoulos A, Valant C. Ligand bias at the muscarinic acetylcholine receptor family: opportunities and challenges. *Neuropharmacology*. 2024;258:110092. doi:10.1016/j.neuropharm.2024.110092
38. **Palma JA. Muscarinic control of cardiovascular function in humans: a review of current clinical evidence. *Clin Auton Res*. 2024;34(1):31-44. doi:10.1007/s10286-024-01016-5**
39. den Hollander LS, IJzerman AP, Heitman LH. Pharmacological characterization of allosteric modulators: A case for chemokine receptors. *Med Res Rev*. 2024;44(5):2291-2306. doi:10.1002/med.22043
40. Shen S, Zhao C, Wu C, Sun S, Li Z, Yan W, Shao Z. Allosteric modulation of G protein-coupled receptor signaling. *Front Endocrinol*. 2023;14:1137604. doi:10.3389/fendo.2023.1137604
41. Franco R, Rivas-Santisteban R, Reyes-Resina I, Navarro G. The old and new visions of biased agonism through the prism of adenosine receptor signaling and receptor/receptor and receptor/protein interactions. *Front Pharmacol*. 2021;11:628601. doi:10.3389/fphar.2020.628601
42. **Napolitano S, Martini G, Ciardiello D, et al. Targeting the EGFR signalling pathway in metastatic colorectal cancer. *Lancet Gastroenterol Hepatol*. 2024;9(8):664-676. doi:10.1016/S2468-1253(24)00123-4**
43. Qu X, Hamidi H, Johnson RM, Sokol ES, Lin E, Eng C, et al. Ligand-activated EGFR/MAPK signaling but not PI3K, are key resistance mechanisms to EGFR-therapy in colorectal cancer. *Nat*

- Commun.* 2025;16:4332.
doi:10.1038/s41467-025-59588-3
44. Abbas N, Mourad M, Smailly H, Al Mahmasani L, Shamseddine A. EGFR signaling in colorectal cancer: novel therapeutic strategies, predictive biomarkers, and counteracting treatment resistance. *Int J Mol Sci.* 2026;27(7):3265. doi:10.3390/ijms27073265
 45. Rosemond E, Rossi M, McMillin SM, Scarselli M, Donaldson JG, Wess J. Regulation of M3 muscarinic receptor expression and function by transmembrane protein 147. *Mol Pharmacol.* 2011;79(2):251-261. doi:10.1124/mol.110.067363
 46. **Sadybekov AV, Katritch V. Computational approaches streamlining drug discovery. *Nature.* 2023;616(7959):673-685. doi:10.1038/s41586-023-05905-z**
 47. Lobbes LA, Schütze MA, Drosier R, Arndt M, Pozios I, Lauscher JC, et al. Muscarinic acetylcholine receptor M3 expression and survival in human colorectal carcinoma—an unexpected correlation to guide future treatment? *Int J Mol Sci.* 2023;24(9):8198. doi:10.3390/ijms24098198
 48. George DM, Lakshminarayanan M, Lakshmanan A. Immunohistochemical expression of mismatch repair proteins in colorectal carcinomas with histopathological correlation in a quaternary care centre in South India. *Apollo Med.* 2025;22(5):389-395. doi:10.1177/09760016241303575