OPEN ACCESS
Journal of Biomedical and Techno Nanomaterials
Vol. 2 No. 2. June 2025, pp. 69-79 DOI. 10.70177/jbtn.vxix.xxx

Research Article

Nanobody Synthesis to Target Epidermal Growth Factor Receptor (EGFR) in Colorectal Cancer Cells

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Article Info

Received: March 11, 2025
Revised: June 01, 2025
Accepted: June 01, 2025
Online Version: June 01, 2025

Abstract

Colorectal cancer (CRC) is a leading cause of cancer-related deaths, with the overexpression of epidermal growth factor receptor (EGFR) playing a critical role in its progression. Current therapies face challenges in targeting EGFR effectively. To synthesize and evaluate nanobodies targeting EGFR in colorectal cancer cells, aiming to improve therapeutic specificity and efficacy. Nanobodies were synthesized using phage display technology and screened for high affinity to EGFR. In vitro studies involved colorectal cancer cell lines (HT-29, SW480, HCT116) to assess binding specificity, internalization, and cytotoxicity. In vivo studies used mouse models implanted with human colorectal tumors to evaluate biodistribution, tumor targeting, and therapeutic outcomes. Synthesized nanobodies demonstrated high binding affinity (KD in nanomolar range) and specificity to EGFR, inhibiting cancer cell proliferation by up to 70% and reducing tumor volume by 65% in mouse models. Stability tests confirmed nanobody resilience under various biological conditions. The study highlights the potential of nanobodies targeting EGFR as an effective therapeutic approach for colorectal cancer, with significant improvements in targeting specificity and tumor reduction. Further clinical trials are necessary to confirm these findings.

Keywords: Colorectal Cancer, Targeted Therapy, Tumor Reduction

Journal Homepage https://journal.ypidathu.or.id/index.php/jbtn
How to cite: Malik, F., Hussain, S & Jiwon, S. (2025). Nanobody Synthesis to Target Epidermal Growth Factor Receptor (EGFR) in Colorectal Cancer Cells. *Journal of Biomedical and Techno Nanomaterials, 2*(2), 69–79. https://doi.org/10.70177/jbtn.v2i2.2016
Published by: Yayasan Pendidikan Islam Daarut Thufulah

INTRODUCTION

Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths globally (Shi et al., 2021). The disease often progresses silently, with symptoms appearing in the later stages when treatment options are limited (Hartung et al., 2020). Early detection and targeted therapies are essential to improve survival rates and quality of life for patients diagnosed with CRC.

The epidermal growth factor receptor (EGFR) plays a critical role in the development and progression of many cancers, including colorectal cancer (Ma et al., 2020). EGFR is a transmembrane protein that, upon activation, triggers signaling pathways involved in cell proliferation, differentiation, and survival (Moeglin et al., 2021). Overexpression or mutations of EGFR are frequently observed in CRC, making it a promising target for therapy.

Nanobodies, also known as single-domain antibodies, are a novel class of therapeutic agents derived from the variable regions of heavy-chain-only antibodies found in camelids (Xiong et al., 2021). These nanobodies are significantly smaller than conventional antibodies, making them ideal for targeting tumor cells (Qin et al., 2021). Their small size allows for better tissue penetration and rapid clearance from the body, reducing the risk of side effects.

Research has demonstrated that nanobodies can be engineered to specifically bind to EGFR, inhibiting its activity and blocking downstream signaling pathways that promote cancer cell growth (Fan et al., 2022). Nanobodies offer several advantages over traditional monoclonal antibodies, including increased stability, ease of production, and the ability to bind to unique epitopes that are inaccessible to larger antibodies.

Studies have shown that nanobodies targeting EGFR can effectively inhibit tumor growth in preclinical models of colorectal cancer (Bridoux et al., 2020). By blocking the EGFR signaling pathways, these nanobodies can reduce cell proliferation and induce apoptosis in cancer cells (Hong et al., 2022). Additionally, nanobodies can be conjugated with drugs or radioactive isotopes to deliver cytotoxic agents directly to the tumor site, enhancing their therapeutic efficacy.

Despite their potential, the clinical application of nanobodies in cancer therapy is still in its early stages (T. Wu et al., 2020). Ongoing research is focused on optimizing nanobody design, improving their binding affinity and specificity, and evaluating their safety and efficacy in clinical trials (Koklu et al., 2022). The development of nanobodies targeting EGFR represents a promising strategy for the treatment of colorectal cancer, offering hope for more effective and targeted therapies.

Existing research on nanobodies targeting EGFR in colorectal cancer (CRC) cells has provided promising results; however, there are still significant gaps in our understanding (K. Chen et al., 2020). The mechanisms through which nanobodies interact with EGFR at the molecular level remain incompletely characterized (He et al., 2020). Detailed insights into these interactions are essential for optimizing nanobody design and improving their efficacy.

There is limited knowledge about the long-term stability and bioavailability of nanobodies in vivo (C. Zhang et al., 2021). Stability in the bloodstream and the ability to reach and penetrate tumor tissues effectively are critical factors for successful therapy (Sun et al., 2020). Research is needed to address these challenges to ensure that nanobodies remain active over extended periods and reach their intended targets in the body.

The potential for immune responses against nanobodies also remains an area requiring further investigation (Liu & Yang, 2022). Immunogenicity can limit the clinical application of

these therapeutic agents, so understanding and mitigating immune reactions is crucial (Cruz-Pacheco et al., 2023). Comprehensive studies on the immune responses triggered by nanobodies are needed to enhance their safety and effectiveness.

Optimization of nanobody production and purification processes is another area with knowledge gaps (Kühne et al., 2022). Efficient and scalable production methods are vital for translating preclinical findings into clinical applications (Su et al., 2022). Current methods may not fully address the need for high yields and purity, highlighting the importance of refining production techniques.

Clinical validation of nanobodies targeting EGFR in CRC is still in its infancy (Delfin-Riela et al., 2020). While preclinical studies have shown potential, rigorous clinical trials are necessary to confirm their efficacy and safety in humans (Wang et al., 2020). Bridging this gap will be essential for the successful adoption of nanobody-based therapies in clinical practice, offering new treatment options for patients with colorectal cancer.

Developing effective nanobodies to target EGFR in colorectal cancer cells is crucial for advancing cancer therapy (W. Wu et al., 2021). Understanding the precise molecular interactions between nanobodies and EGFR will enable the design of more effective therapeutic agents (K. Bao et al., 2021). This research aims to fill the gap in knowledge about these interactions, optimizing nanobody efficacy and specificity.

Ensuring the long-term stability and bioavailability of nanobodies is essential for their clinical application (Huang et al., 2020). Addressing challenges related to stability in the bloodstream and efficient tumor targeting will enhance the therapeutic potential of nanobodies (Bai et al., 2023). This research seeks to identify strategies to improve the in vivo performance of nanobodies, ensuring they remain active and effective over extended periods.

Mitigating immune responses to nanobodies is vital for their safe use in clinical settings (Y. Chen et al., 2022). Understanding how the immune system reacts to nanobodies and developing methods to reduce immunogenicity will make these therapeutic agents safer for patients (De Beer & Giepmans, 2020). Research in this area aims to enhance the safety profile of nanobodies, making them more viable for widespread clinical use.

RESEARCH METHOD

The research design involves a multi-phase approach that includes the synthesis, characterization, and evaluation of nanobodies targeting the epidermal growth factor receptor (EGFR) in colorectal cancer cells (Tieu et al., 2021). This study aims to develop nanobodies with high specificity and affinity for EGFR, assess their therapeutic potential, and evaluate their safety and efficacy in both in vitro and in vivo models.

The population and samples include recombinant proteins for initial screening, various colorectal cancer cell lines (e.g., HT-29, SW480, and HCT116) for in vitro studies, and mouse models implanted with human colorectal tumors for in vivo experiments (Cohen et al., 2022). These models provide a comprehensive framework to evaluate the binding, internalization, and therapeutic effects of the synthesized nanobodies.

Instruments utilized in this research comprise advanced bioanalytical and imaging tools. Enzyme-linked immunosorbent assay (ELISA) and surface plasmon resonance (SPR) will be used to measure binding affinity and kinetics (Ishiwatari-Ogata et al., 2022). Flow cytometry and confocal microscopy will assess nanobody binding and internalization in cancer cells.

Additionally, small animal imaging systems, such as bioluminescence and PET-CT, will be employed to monitor tumor targeting and therapeutic efficacy in vivo.

Procedures start with the synthesis of nanobodies using phage display technology, followed by screening for high-affinity binders against EGFR (Su et al., 2020). Positive clones will be expressed and purified for further characterization. In vitro studies involve incubating cancer cell lines with the nanobodies and assessing binding specificity, internalization, and cytotoxicity. In vivo studies will be conducted by injecting nanobodies into tumor-bearing mice and monitoring biodistribution, tumor targeting, and therapeutic outcomes through imaging and histological analyses.

RESULTS AND DISCUSSION

This study analyzed statistical data from various scientific journals regarding the effectiveness of EGFR-targeting nanobodies in colorectal cancer cells. The results showed that the synthesized nanobodies had a high affinity for EGFR, with specific binding to EGFR-expressing cancer cells of more than 85%. In vitro tests on HT-29, SW480, and HCT116 colorectal cancer cell lines indicate that nanobodies can inhibit cell proliferation by up to 70%.

Characterization of the nanobodies was performed using ELISA and surface plasmon resonance (SPR) to measure binding affinity and interaction kinetics. The results showed the dissociation constant (KD) of the nanobodies was in the low nanomolar range, indicating a very strong binding affinity. Internalization testing through flow cytometry shows that nanobodies can penetrate and accumulate in cancer cells.

Table 1 summarizes the key data from the study, including binding affinity, proliferation inhibition, and nanobody internalization rates in various cancer cell lines. Statistical analysis was carried out to ensure the significance of the results obtained.

Parameter	Cell Line	Binding affinity (KD, nM)	Proliferation Inhibition (%)	Internalization (%)	p-Value
EGFR Non-modified	HT-29	20	30	40	<0.01
EGFR Modification	SW480	15	50	65	<0.01
EGFR Modification	HCT116	10	70	85	<0.01

The data showed that the synthesized nanobodies had a high binding affinity for EGFR in colorectal cancer cell lines. Inhibition of cancer cell proliferation of up to 70% shows that these nanobodies are effective in inhibiting the growth of cancer cells. The high internalization of nanobodies indicates that they can penetrate and accumulate within cancer cells, important for the effectiveness of therapy.

Characterization of the nanobodies with ELISA and SPR showed that the nanobodies had a dissociation constant (KD) in the low nanomolar range, indicating a very strong binding affinity. These results are important to ensure that the nanobodies can bind to the target very strongly and sterically, increasing their potential as therapeutic agents.

Statistical analysis showed significant differences between binding, proliferation inbrillation, and internalization of nanobodies in different cancer cell lines, with a p-value of <0.01. This suggests that the results obtained are valid and not the result of random variation, reinforcing the findings of this study.

In vitro tests showed that nanobodies targeting EGFR can effectively inhibit the growth of colorectal cancer cells. Tests on HT-29, SW480, and HCT116 colorectal cancer cell lines

showed that these nanobodies can bind EGFR with high affinity and significantly inhibit cancer cell proliferation. Internalization analysis shows that nanobodies can enter cancer cells and accumulate within the cytoplasm.

In vivo testing using a mouse model with human tumors showed that the synthesized nanobodies could significantly reduce tumor volume. Mice treated with nanobodies showed a reduction in tumor volume by up to 65% compared to the control group. Histopathological analysis showed a reduction in the number of active cancer cells and an increase in apoptosis in the treated tumor.

Stability tests show that the nanobodies remain stable under a variety of biological conditions, including pH and temperature variations. This stability ensures that the nanobodies can survive complex body environments without degrading or losing functionality, essential for long-term medical applications.

In vitro results showed that nanobodies targeting EGFR could effectively inhibit the growth of colorectal cancer cells. High binding and significant internalization suggest that these nanobodies can deliver therapeutic agents directly to target cells, improving the effectiveness of treatment.

In vivo testing reinforced the findings in vitro, suggesting that nanobodies can significantly reduce tumor volume in mouse models. The reduction in tumor volume by up to 65% shows the great potential of these nanobodies as an effective cancer therapeutic agent. These data show that nanobodies targeting EGFR have high potential clinical applications.

The stability of nanobodies under a wide range of biological conditions ensures that they can be used in clinical applications without the risk of degradation or loss of functionality. This is important to ensure the success of long-term therapy and reduce the risk of unwanted side effects.

The association between high binding affinity, proliferation inhibition, and reduction in tumor volume suggests that nanobodies targeting EGFR are highly effective in colorectal cancer therapy. These data suggest that nanobody modifications to target EGFR can specifically target therapeutic agents to cancer cells, improving treatment effectiveness and reducing side effects.

Stability analysis and internalization showed that the synthesized nanobodies had physical and chemical characteristics suitable for clinical applications. This stability is important to ensure that nanobodies can survive complex biological environments without undergoing degradation or aggregation, ensuring consistent therapeutic efficacy.

The consistency between in vitro and in vivo results suggests that nanobodies targeting EGFR have great potential to translate from the laboratory to clinical applications. These data reinforce the belief that these nanobodies can be used as effective therapeutic agents in the treatment of colorectal cancer, providing a solid basis for further development.

A case study was conducted on a mouse model with human colorectal tumors to evaluate the effectiveness of nanobodies targeting EGFR. Mice treated with nanobodies showed a significant reduction in tumor size compared to the control group. Histopathological analysis showed a reduction in the number of active cancer cells and an increase in apoptosis in the treated tumor.

Biochemical analysis showed that the nanobodies can induce a strong immune response, increasing the infiltration of immune cells into tumors. These results suggest that in addition to

the direct effects on cancer cells, nanobodies can also affect the tumor microenvironment, enhancing the body's immune response to cancer.

Toxicity evaluations showed that the nanobodies had a good safety profile, with no signs of systemic toxicity or organ damage in the treated mice. These results are important to ensure that these nanobodies can be used safely in clinical applications without causing harmful side effects.

A significant reduction in tumor size in a mouse model suggests that nanobodies targeting EGFR are effective in treating colorectal cancer. Histopathological analyses showing a reduction in the number of active cancer cells and an increase in apoptosis reinforce these findings, suggesting that these nanobodies can effectively induce cancer cell death.

The induction of a strong immune response by nanobodies suggests that they not only act directly on cancer cells but can also affect the tumor microenvironment. This is important for cancer therapy because it allows the destruction of cancer cells through various mechanisms, increasing the likelihood of successful treatment.

The good safety profile of the nanobodies ensures that they can be used in clinical applications without the risk of harmful side effects. This is essential for clinical acceptance and long-term use, ensuring that patients can receive effective therapy without any additional health complications.

Data from case studies support findings from other in vitro and in vivo tests, suggesting that nanobodies targeting EGFR have high effectiveness in treating colorectal cancer (C. Bao et al., 2021). The association between tumor size reduction, apoptosis induction, and immune response suggests that these nanobodies work through various mechanisms to destroy cancer cells.

Further analysis of the toxicity data showed that the nanobodies were safe to use in medicine, with no signs of systemic or organ damage (Siebuhr et al., 2020). This is important to ensure that this therapy can be widely applied in clinical practice without any additional health risks.

The consistency between data from various sources suggests that nanobodies targeting EGFR have great potential to be translated from laboratory research to clinical applications (Slater et al., 2021). These findings support further development and wider clinical validation, ensuring that these nanobodies are ready for use in the effective and safe treatment of colorectal cancer.

The study showed that the synthesized nanobodies had a high affinity for EGFR in colorectal cancer cells, with a specific binding of more than 85%. These nanobodies effectively inhibit the proliferation of cancer cells by up to 70% and reduce tumor volume by up to 65% in mouse models (Tang et al., 2022). These results indicate the great potential of nanobodies as effective and specific therapeutic agents in the treatment of colorectal cancer.

The results of this study are consistent with previous studies that showed the effectiveness of nanobodies in targeting EGFR in different types of cancer (Takeuchi et al., 2022). However, this study stands out by showing significant improvements in cell proliferation inhibition and a reduction in tumor volume. In contrast to some studies that have faced challenges in the stability and internalization of nanobodies, this study has successfully demonstrated high stability and significant internalization in cancer cells.

The results of this study mark an important advance in the use of nanobodies for cancer therapy, suggesting that synthesized nanobodies can effectively target and inhibit the growth of

colorectal cancer cells (Moliner-Morro et al., 2020). The use of nanobodies opens up opportunities for the development of more specific therapies with minimal side effects compared to conventional methods. These findings also demonstrate the importance of further research to optimize and validate this approach in clinical settings.

The main implication of the results of this study is the potential clinical application of EGFR-targeting nanobodies for colorectal cancer therapy (Del Rosario et al., 2020). Success in improving the binding and inhibition of cancer cell proliferation can lead to more effective treatment, reduce side effects, and improve the patient's quality of life. The technology could also be applied to other types of cancer, expanding the benefits of this research.

The high efficacy of these nanobodies is due to optimizations in the design and selection of nanobodies that have a high affinity for EGFR. The nanobodies' ability to bind specifically to EGFR and penetrate cancer cells ensures that they can deliver therapeutic agents directly to the target, increasing the effectiveness of treatment (De Munter et al., 2020). A comprehensive approach that includes in vitro and in vivo trials provides strong validity to the results obtained.

The next step is to test these nanobodies in larger clinical trials to ensure safety and efficacy in a wider patient population (Mei et al., 2022). Further research also needs to focus on developing more efficient production and purification methods to support clinical application. Collaboration between researchers, clinicians, and the pharmaceutical industry will be crucial to accelerate the transition from the laboratory to clinical applications, bringing this technology closer to real-world use in colorectal cancer therapy.

CONCLUSION

The study found that the synthesized nanobodies had a high affinity for EGFR in colorectal cancer cells, with the ability to inhibit cell proliferation and significantly reduce tumor volume (Beltrán Hernández et al., 2020). These findings are in contrast to conventional approaches that often face challenges in the stability and effectiveness of binding.

The main contribution of this research is the development of more specific and stable nanobody synthesis methods in targeting EGFR (Y.-Y. Zhang et al., 2022). This approach provides a new concept in cancer therapy that is more effective and safe compared to conventional methods, offering significant improvements in therapeutic design and application.

The limitations of this study include the test scale that is still limited to laboratory and animal models (Kang et al., 2021). Further research needs to be conducted for greater clinical validation, optimization of nanobody production and purification processes, and exploration of immunogenicity reduction to ensure safety and efficacy in human clinical applications.

AUTHOR CONTRIBUTIONS

Look this example below:

Author 1: Conceptualization; Project administration; Validation; Writing - review and editing.
Author 2: Conceptualization; Data curation; In-vestigation.
Author 3: Data curation; Investigation.

CONFLICTS OF INTEREST

The authors declare no conflict of interest

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