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Inhibition of Cancer Causing Genes Through the Delivery of OmoMyc in Anti-Myc Therapy: a Systematic Review

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Inhibition of Cancer Causing Genes Through the Delivery of OmoMyc in Anti-Myc Therapy: A Systematic Review

by

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ABSTRACT

A systematic review of the available studies on the interference of OmoMyc with Myc's function in cancerous cells is presented. Myc is a transcription factor that regulates cellular processes such as apoptosis, proliferation, and differentiation. However, Myc is often overexpressed in a variety of cancers, resulting in abnormal growth of cancer cells. Although the inhibition of Myc has been highly desired, it remains a challenge due to its undruggable characteristics. Attempts to inhibit Myc have involved the usage of small-molecules, but these attempts have failed, causing adverse effects and incomplete inhibition of Myc. Despite promising preclinical studies of OmoMyc, it is only considered as a proof of principle due to the difficulties to deliver into cells. The results of this systematic review are discussed in hopes of overcoming difficulties in the delivery of OmoMyc *in vitro* transcribed mRNA (IVT-mRNA) in efforts to inhibit the overexpression of Myc.

DEDICATION

To my parents, my teachers, my friends whose immeasurable support have gotten me to where I am today and one step closer to my dreams. From the late nights studying, to the early mornings going to lectures, I want to thank you all for being there for me every step of the way.

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INTRODUCTION

With 1.7 million new cases and 0.6 million deaths in the last year, cancer has become one of the most devastating causes of death in the United States (Siegel et al. 2019). From the efforts to understand its cause, we now know that cancer occurs due to changes in our genes. These cancer-causing genes are termed oncogenes. Myc is one of the major proto-oncogenes, which makes it a desirable target for cancer treatment. In normal cells, Myc is responsible for the regulation of various target genes that work together in the transcription process and cell proliferation. In combination with Myc's partner protein, Max, the activation and repression of many target genes are regulated (Kalkat et al. 2017). The Myc/Max complex is composed of bHLH-Zip DNA binding proteins which recognizes the E-box sequences in DNA to activate transcription (Cascon et al. 2012, Valovka et al. 2013). However, the expression of Myc is often amplified in many types of cancers. This amplification results in increased Myc RNA expression, leading to cancer cell proliferation, tumor growth, and angiogenesis, among other cellular processes. These are the primary characteristics of cancers.

Previous attempts to inhibit Myc have failed. The main reason for difficulties targeting Myc is due to its structure, Myc's role in healthy cells, and Myc's location in the cell. Due to Myc's structure, researchers have had difficulties in finding sites that are commonly used by pharmacological strategies. Since Myc is found in healthy cells,

utilizing small molecules results in a high risk of side effects on the healthy cells. In addition, Myc is located in the nucleus, making it difficult for access by small molecules and antibodies.

The discovery of OmoMyc has aided efforts in finding an efficient solution. OmoMyc is a dominant-negative form of Myc, differing in four amino acids, E57T, E64I, R70Q, and R71N (Soucek et al. 1998). The four changes altered the transcriptional effects of Myc by competitive inhibition. The competitive inhibition interferes with the heterodimerization of Myc/Max, which alters the function of Myc (Sauvino et al. 2011). OmoMyc also prevents the binding of Myc/MAX complex to promoter E-boxes, which inactivates Myc-derived transcriptions.

Preclinical studies have shown that OmoMyc is successful in the inhibition of Myc in transgenic mouse models. In these mouse models, OmoMyc inhibited Myc resulting in tumor growth inhibition and eradication (Soucek et al. 2008). These models showed mild, reversible side effects, validating the safety of OmoMyc. Yet, clinical transition of OmoMyc remains a challenge because firstly, OmoMyc is too big to cross the cell membrane. Secondly, in physiological conditions, due to proteinase in plasma, proteins can be degraded. Thirdly, proteins can be immunogenic. For these reasons, OmoMyc is only considered as proof of principle.

Recent studies have examined the effects of anchoring small peptides that are known to assist the cellular entry of OmoMyc. However, this approach still requires the administration of the OmoMyc protein in a large amount due to limited delivery efficiency. The degradation of OmoMyc and its potential immunogenicity still remains. We examined that the delivery of OmoMyc in a form of *in vitro* transcribed (IVT) mRNA would enhance the intracellular delivery of OmoMyc protein, therefore, resulting in a greater inhibition of Myc. Considering that one mRNA molecule produces multiple proteins, this strategy would inhibit Myc more efficiently than delivery of OmoMyc protein. In order to protect and deliver OmoMyc mRNA, lipid-based nanoparticles, which are clinically relevant delivery vectors, will be examined. Using lipid-based nanoparticles as a vector for mRNA delivery reduces the risks of degradation and immunogenicity in plasma. We anticipate to demonstrate the viability of OmoMyc in lipid-based mRNA therapy for the future of cancer therapeutics.

METHODS

Sample of Studies

A systematic review of studies available by 2008 from biomedical journals such as *Cell*, *Nature*, and *Science Translational Medicine* are conducted. From this, we propose to examine the intracellular delivery of OmoMyc IVT-mRNA using lipid-based nanoparticles, and the Myc inhibition by translated proteins in cancer cells overexpressing Myc. Keywords, *OmoMyc*, *Myc*, *cancer therapeutics*, *in vitro transcribed (IVT) mRNA*, *mRNA therapy*, *lipid-based nanoparticles*, and *cancer therapeutics* were used on these computerized databases. Since researchers often use the terms, *vectors* and *delivery vectors*, we conducted searches using these substitute terms as well.

After identifying the main body of reports, we examined the references of these reports to obtain other relevant reviews that were not included in our initial database search. From these reports, we examined the journal volumes that they were in.

Studies that tested the delivery and effect of OmoMyc on any type of mouse models or xenograft models will be included. Relevant articles were extracted from several online databases using predefined data fields, indicating the year of the study, the topic of the study, and the effectiveness of the study.

Selection Criteria

We used the following criteria to select studies for inclusion in the systematic review.

1. We only included studies whose main focus involved Myc as a therapeutic target. Thus, we excluded studies in which Myc only played a minor role or was briefly mentioned.
2. We included studies who mentioned the three Myc gene family members, MYC, MYCN, and MYCL. These three families are linked to Myc inhibitory strategies.
3. We only included studies published by 2008, as technology, cancer therapeutics mechanisms, and knowledge of Myc's properties rapidly change over time.
4. We included studies who delivered OmoMyc, and/or expressed OmoMyc in cancerous cells, and/or tested the efficacy of OmoMyc.
5. We included studies that used Polymerase Chain Reactions (PCR) and Western Blot analysis.
6. We included studies who delivered OmoMyc in protein form or in mRNA form.
7. We included studies in peer-reviewed journals and notable academic articles in our search.

Our selection criteria led to a database containing 128 studies involving the delivery and efficacy of OmoMyc in its protein form and in its mRNA form. Of the 128 studies, 100 were peer reviewed journals. The 128 studies consisted of 117 articles, 6 dissertations and theses, 3 newspaper articles, and 2 reviews. The main keywords that were shown in the articles were *proto-oncogene proteins*, *cancer*, *Myc protein*, *gene expression*, *transcription factors*, and *cell proliferation*.

DATA

Selected Studies

Of the resulting database, we selected five studies discussing Myc inhibition, OmoMyc functions, and the utilization of lipid encapsulation for the delivery of OmoMyc.

I. MYC INHIBITION

Myc is a basic helix-loop-helix leucine zipper transcription factor that regulates the expression of the intracellular and extracellular processes that are necessary for growth of somatic cells (Soucek et al. 2008). Due to this, the inhibition of Myc is one of the main pharmacological approaches in cancer therapeutics. Despite the potential success of inhibiting Myc, there are detrimental side effects that would inhibit the proliferation of normal tissues (Soucek et al. 2008).

When genetically modelling the therapeutic impact and the side effects of Myc inhibition in a preclinical mouse model of Ras-induced lung adenocarcinomas, studies have shown that inhibition of Myc has various detrimental side effects. However, these effects are reversible and tolerable. Complete inhibition of Myc would have negative effects on tissues such as the gastrointestinal tract, skin, and bone marrow (Soucek et al. 2008). Challenges such as the irreversible side effects

on healthy somatic cells have led to concerns about Myc inhibition as a way to treat cancer.

In the Soucek study, Omomyc was conditionally expressed using the cytomegalovirus (CMV) early promoter. CMV is associated with high levels of expression in multiple tissue types (Soucek et al. 2008). The coding sequence for OmoMyc was placed downstream of a tetracycline-responsive promoter element (TRE). Mouse models were utilized in this study as well. Mice with the TRE-Omomyc transgene were then crossed. From there, a polymerase chain reaction with reverse transcription (RT-PCR) ensured that OmoMyc expression was in the tested tissues.

To assess the inhibition of Myc's function using OmoMyc, the study used a lung cancer mouse model, where tumorigenesis is initiated by activation of endogenous Kras. Kras are common mutations in human non-small-cell lung cancers (Soucek et al. 2008). To analyze the contribution of endogenous Myc, TRE-OmoMyc was crossed. CMVrtTA mice and OmoMyc induced were maintained for four weeks. Lesions were absent from all mice expressing KrasG12D and OmoMyc. As a result, the expression of OmoMyc suppressed the fraction of cell proliferation. Apoptosis in the residual lesions of mice expressing Omomyc was observed. At the end of the study, the lung morphology, cell

proliferation, and apoptosis of the mice were assessed immunohistochemically. The mice expressing KrasG12D without OmoMyc developed multiple hyperplasias (Soucek et al. 2008). This study suggested that endogenous Myc is required to maintain the survival of KrasG12D induced lung tumors. This study confirmed that Myc has a crucial role in proliferation of healthy and cancerous cells, reiterating that Myc's inhibition would cause adverse side effects. Inhibition of Myc led to shrinkage of lesions, reduction of lesion multiplicity, significant apoptosis in residual lesions, and reduction of proliferating cells (Soucek et al. 2008). Another trial in the study showed that Myc inhibition induced no apoptosis and that OmoMyc expression was not harmful to the mice. As a whole, the study showed that Myc inhibition showed rapid regression of both incipient and established lung tumors, and affects normal regenerating tissues (Soucek et al. 2008). The study showed that OmoMyc could be a potential Myc inhibitor.

Summary of the Findings of the Soucek Study

1. Myc inhibition prompts rapid regression of incipient and established lung tumors. This indicates endogenous Myc's role in the maintenance of Ras-dependent tumors *in vivo* (Soucek et al. 2008).
2. Systemic Myc inhibition has effects on normal generating tissue (Soucek et al. 2008). This again emphasizes the side effects of

Myc inhibition and why OmoMyc is currently considered as a proof principle. These side effects may be reversible, but there is still some uncertainty in OmoMyc's efficacy in the inhibition of Myc.

3. OmoMyc has potential to be a way to inhibit Myc's function in cancerous cells.

II. TESTING THE EFFICACY OF OMOMYC

OmoMyc has been highly sought after in the inhibition of Myc. Current studies have shown that OmoMyc is a highly stable homodimer and forms a tight protein/DNA complex (Jung et al. 2017). Knowing this aids in efforts to understand OmoMyc gene expression regulation. Studies have observed the expression of OmoMyc and have purified the OmoMyc recombinant protein. It is also observed that when binding to the DNA, it forms a scissor-like structure at the E-box. This is done with stabilization through the phosphate-backbone interactions with DNA.

In the Jung study, the protein/DNA complex of OmoMyc was compared to the Myc/Max DNA complex, indicating that the basic region of both complexes have the same contacts to DNA. This suggests that OmoMyc has the capabilities of

competing with the Myc/Max complex for E-box binding (Jung et al. 2017). Other OmoMyc functionalities examined by this study includes attenuation of oncogenic Myc-dependent gene expression and suppression of tumor growth. This study focuses on the reproduction of OmoMyc's efficacy with small molecule inhibitors and the analysis of OmoMyc's target genes *in vivo*. Through expression and purification of recombinant OmoMyc, and Myc and Max protein, crystallization of OmoMyc, and assays, "findings suggest that individual genes encoding rate-limiting proteins involved in basic cell biological processes respond to the difference between physiological and supra-physiological MYC levels and that OmoMyc specifically inhibits tumor cell growth as it suppresses expression of this group of genes" (Jung et al. 2017).

Summary of the Findings of the Jung Study

1. OmoMyc is a highly stable homodimer and forms a tight protein/DNA complex (Jung et al. 2017). Tight protein/DNA interactions are favorable.
2. OmoMyc blunts promoter invasion by oncogenic Myc levels (Jung et al. 2017). This reduces the gene expression of Myc-dependent tumors.

On a similar basis to the Jung study, a 2011 study by Savino indicated that OmoMyc selectively targets the Myc interactome, affects the transactivation and repression by influencing Myc binding to target gene promoters, and affects proliferation and survival of cells in culture. OmoMyc prevents Myc binding to promoter E-boxes. This study utilized Western Blot analysis, immunoblotting, and mouse models. Indication that OmoMyc selectively targets the Myc interactome was shown by immunoprecipitations on cells expressing Myc. The Myc interactome consists of interaction with the bHLHZip protein Max. This interaction is crucial to Myc's function.

In efforts to investigate OmoMyc effects in gene regulation, luciferase reporter and chromatin immunoprecipitation (ChIP) assays were conducted on two genes encoding the nucleolar protein nucleolin and the cyclin-dependent kinase inhibitor p21. These represent the "direct targets of Myc mediated activation and repression" (Sauvino et. al 2011).

Summary of the Findings of the Savino Study

1. OmoMyc selectively targets the Myc interactome (Savino et al. 2011). Selective targeting increases the effectiveness and efficiency of drug delivery.

2. OmoMyc differentially affects transactivation and repression by its interaction with the Myc/Max complex. OmoMyc affects Myc binding to target gene promoters (Savino et al. 2011).
3. OmoMyc influences proliferation and cell survival in culture (Savino et al. 2011).

The third study retrieved from the database also utilized immunoprecipitations, Western blotting, and mouse models to observe the efficacy and mechanisms of OmoMyc in the inhibition of the Myc oncogene. The study shows that treating cells with recombinant OmoMyc leads to a reduction in the levels of the Myc protein (Demma et al. 2019). Proximity ligation assays (PLAs) have shown that OmoMyc can interact with the Myc complex. This interaction is the formation of dimers with both Max and differentially labeled OmoMyc monomers in the cell (Demma et al. 2019). Translation of ribosome affinity purification (TRAP) and RNA immunoprecipitation (RIP) experiments suggest that Myc, OmoMyc , and Max can interact with ribosomes and Max RNA in conditions where ribosomes are intact. This is an indication of cotranslational dimerization of proteins with Max (Demma et al. 2019).

The Demma study shows that recombinant OmoMyc inhibits cancer cell proliferation and affects MYC-mediated transcriptions (Demma et al. 2019). After

purification, recombinant OmoMyc was used to treat cell lines where Myc was amplified or stabilized. Effects on lymphoma cell lines containing high levels of Myc have shown sensitivity to OmoMyc. In contrast, lymphoma cell lines containing low levels of Myc are insensitive to OmoMyc.

In addition to demonstrating how OmoMyc inhibits cancer cell proliferation, the study discussed how OmoMyc binds to the Myc/Max complex. They utilized a biotinylated OmoMyc to immunoprecipitate Myc and Max from cells. After treatment with two different amounts of OmoMyc, 2.5 μM or 10 μM, the cells were then lysed and immunoprecipitation with anti-Myc, anti-Myc, and streptavidin were carried out. Western blotting was used to show that when the cells were treated with OmoMyc, a reduction in the amount of Myc that could be immunoprecipitated with Max occured (Demma et al. 2019). Myc and Max were bound to biotinylated OmoMyc. This showed successful interaction of OmoMyc with Myc and Max in cells and the effect of OmoMyc on Myc stability and protein levels. OmoMyc was also observed to have directly targeted and localized to the nucleolus in cells. Most importantly, OmoMyc decreased the overall level of Myc in the cell.

Summary of the Findings of the Demma Study

1. Recombinant OmoMyc inhibits cancer cell proliferation and affects Myc-mediated transcription (Demma et al. 2019).
2. OmoMyc interacts with the Myc/Max complex and leads to the destabilization of Myc (Demma et al. 2019).
    a. OmoMyc forms heterodimers with Max in the cells, which bind to promoter regions of Myc target genes (Demma et al. 2019). This shows OmoMyc's success in its interactions with Myc.
3. OmoMyc binds to E-box sequences in DNA and displaces Myc/Max heterodimers (Demma et al. 2019).
4. OmoMyc localizes to the nucleolus in cells (Demma et al. 2019). This is especially important in the inhibition of Myc, as Myc is located in the nucleus of cells, making it challenging to get to.
5. *In vivo*, OmoMyc rapidly distributes to tissues (Demma et al. 2019). This is promising in developing OmoMyc as a method in cancer therapeutics.

III. DELIVERY OF OMOMYC USING LIPID ENCAPSULATION

As shown in the mentioned studies, OmoMyc is a viable option for inhibiting Myc's functions, however, due to side efforts of Myc's inhibitions, OmoMyc is only considered as a proof of concept. Current delivery methods include small molecules, which have not been as successful as researchers hoped. In efforts to understand and develop OmoMyc, we propose to deliver OmoMyc through lipid encapsulation.

One of the main issues with OmoMyc delivery as a protein is that it is bulky and has been difficult to pass through the cell membrane to get to the nucleus, where Myc is located.

The Myc family consists of three related human genes: c-Myc, l-Myc, and n-Myc. The c-Myc transcription factor is dependent on the heterodimerization with Max to control target gene transcription (Pan et al. 2015). Current efforts with small-molecule inhibitors of c-Myc/Max have shown low potency and poor water solubility (Pan et al. 2015). Due to this, these small-molecule inhibitors are unsuitable for *in vivo*. Proposed is a new lipid-based strategy for delivering oncogenic c-Myc inhibitors for targeting in melanoma cells (Pan et al. 2015).

An Sn-2 lipase-labile Myc inhibitor prodrug was synthesized and was included in two αv β3-targeted nanoparticle platforms at 20 and 200 nm (Pan et al. 2015). The antiproliferate potency was compared to the lipid-free compound (Pan et al. 2015). Human and mouse melanoma cell lines were observed. The data collected from this study demonstrated a successful nanodelivery of c-Myc inhibitors through lipid-based encapsulation, suggesting their potential to prevent melanoma.

This study suggests that delivery through lipid-based encapsulation could be useful in delivering different Myc targeting therapies. As mentioned previously, lipid based nanoparticles as a vector for mRNA delivery reduced the risk of degradation and immunogenicity of OmoMyc in plasma.

CONCLUSION AND FUTURE WORK

Based on the findings of this systematic review, OmoMyc has the potential to be a viable option for the inhibition of Myc. The inhibition of Myc has adverse side effects, however, better understanding of Myc's functions could lead to a successful development of OmoMyc. For future studies, we hope to be able to test the efficacy of OmoMyc by examining the intracellular delivery of OmoMyc IVT-mRNA using lipid-based nanoparticles. To aid in these efforts, we hope to better understand the Myc inhibition by translated proteins in cancer cells overexpressing Myc.

Branching from this study, we have designed a research plan in hopes of facilitating the development of OmoMyc. The first part of the research plan consists of the synthesis of Omomyc IVT-mRNA. We will subclone the sequence of Omomyc into plasmid DNA, and amplify the engineered plasmid in E. coli. Afterward, the amplified plasmid will be purified, and prepared for the in-vitro transcription. Once we have obtained IVT-mRNA, we will encapsulate it in lipid-based nanoparticles. The second part of the research plan is to deliver the OmoMyc IVT-mRNA into the cell, which will be translated into protein, reaching the nucleus to perform its activities. We will be screening different cancer cell types that are overexpressing Myc to identify the best candidate for the proposed research. Once we identify the desired cancer cell type, we can deliver the OmoMyc IVT-mRNA and observe its activities.

To analyze these activities, Western blot analysis and fluorescent microscopy will be conducted. We anticipate that this new delivery strategy will help prove the feasibility of OmoMyc for the treatment of Myc-driven cancers.

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