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"Data-Driven Innovations: Harnessing Chat GPT-4's Potential for Break throughs in Cancer Treatment"

Dr. Dhruv Subramanian

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ABSTRACT:
Utilizing the ChatGPT-4 AI model, we reevaluated the prevailing hypothesis regarding the silencing of TCTP. Contrary to prior beliefs, silencing TCTP might not necessarily trigger a cascade of downstream effects in the molecular pathway leading to spontaneous regression in Neuroblastoma. While this questions its potential implications for other cancers, such as Oral cancer, the therapeutic targeting of TCTP remains promising, as evidenced in its exploration within Prostate cancer and other malignancies. Further research is warranted to elucidate its full potential.

I. INTRODUCTION:

Translationally Controlled Tumor Protein (TCTP) plays a pivotal role in numerous biological functions, encompassing cell growth, apoptosis, cell cycle progression, and tumor genesis. Its widespread implications underline its significance in the realm of cancer biology. Up until September 2021, there's been increasing interest in targeting TCTP for anti-cancer therapies across various malignancies. However, the overarching question remains: Can silencing TCTP truly instigate spontaneous regression in cancers such as neuroblastoma and oral cancer?

Diving deeper, several aspects warrant attention:
1. **Oncogenic Properties of TCTP**: While TCTP's over expression is tied to multiple cancers, the exact dynamics of how it contributes to tumor genesis remain a subject of intense research. Its participation in pathways like apoptosis, autophagy, and cell cycle modulation suggests that silencing it could hinder tumor progression.

2. **Heterogeneity in Cancer**: The inherent diversity in tumors posits that a single-target approach may not universally prompt spontaneous regression. Adaptability of tumors, allowing them to evade targeted treatments, further complicates the scenario.

3. **Literature Insights**: By 2021, multiple studies showcased the implications of TCTP inhibition in different cancer cell lines. Yet, responses differ considerably between, and even within, cancer subtypes. A meticulous review of contemporary research is essential to glean insights into TCTP's role in neuroblastoma and oral cancer.

4. **Cascade Effects**: Disrupting genes, especially pivotal ones like TCTP, can ripple through molecular pathways. Understanding these ramifications is crucial, given that they can either be therapeutically beneficial or inadvertently harmful.

5. **Balancing Act in Therapy**: Even if silencing TCTP offers therapeutic advantages, gauging the balance—between therapeutic gains and potential adversities to healthy cells—becomes paramount.

6. **Challenges in Gene Silencing**: Actualizing efficient gene silencing, particularly in vivo in solid tumors, is a formidable task. Observations in controlled environments, such as cell cultures or animal models, don't always seamlessly translate to human clinical contexts.

In encapsulation, while TCTP emerges as a promising therapeutic axis in oncology, its silencing doesn't universally assure tumor regression. Comprehensive scientific investigations, meticulous preclinical evaluations, and rigorous clinical trials are imperative to gauge the prospects of targeting TCTP in specific malignancies like neuroblastoma and oral cancer.

II. INTRODUCTION & SPECIFIC AIMS

Neuroblastoma (NB), diagnosed predominantly in the first year of life, exhibits a curious pattern of spontaneous regression in certain patients. This phenomenon has instigated worldwide efforts to understand its underlying mechanisms, as evident from the newly introduced International NB Risk Group Staging System. Japan, having embarked on an extensive mass screening regimen for NB in newborns over past decades, has championed a 'wait-and-see' approach for early-detected NB tumors. While this can occasionally offer a beneficial wait for spontaneous

regression, there are instances where it proves detrimental to the patient's prognosis.

Given the aforementioned challenges, the scientific community's interest has been piqued towards identifying markers and pathways that could provide new therapeutic directions. Enter the Translationally Controlled Tumor Protein (TCTP). Touted as a tumor reversion protein, its efficacy across cancers such as prostate, lung, and colon has been noted. With no existing explorations of TCTP in NB treatment avenues, the logical step forward is to probe its potential role in inducing spontaneous regression or its broader implications for NB.

Further widening the scope of inquiry, there's a burgeoning interest in OLP (Oral Lichen Planus), a chronic inflammatory condition affecting oral mucous membranes. The objective here is to discern markers unique to OLP by fine-tuning the affected genetic pathways, notably those pertinent to TCTP production. By embarking on differential expression studies on healthy versus diseased oral tissues, and leveraging the capabilities of microarrays, the aim is to unveil siRNA targets for OLP interventions. A future direction could also encompass the formulation of a clinical measure, pivoted around TCTP expression, offering prognosis values for OLP, thereby enhancing patient care.

In essence, both NB and OLP present intriguing clinical puzzles. The role of TCTP, with its multifaceted interactions and potential implications, could be the missing piece in these puzzles. Thus, the overarching goal of this research is twofold:

Specific Aim 1: Dive deep into NB, exploring whether TCTP-laden exosomes interact with either the NUMB protein or the Notch 1 receptors.

Specific Aim 2: Navigate the realm of OLP, discerning the impact of TCTP, alongside potential novel gene markers, to refine treatment protocols and enhance prognosis predictions.

With a harmonized focus on both NB and OLP, this proposal endeavors to venture into uncharted territories, aiming to glean insights that could revolutionize treatment paradigms for both conditions.

III. BACKGROUND AND SIGNIFICANCE

Neuroblastoma (NB) is a pediatric cancer that primarily originates in the sympathetic nervous system tissues. Notably, some of these tumors undergo spontaneous regression, yet the driving mechanisms remain a mystery. This research aims to investigate the potential role of Translationally Controlled Tumor Protein (TCTP) in this spontaneous regression, targeting a deeper understanding of the cascade of molecular events that may lead to this phenomenon. One core hypothesis is that TCTP may inhibit the Notch 1 receptor, leading to the modulation of the Notch 1 signaling pathway and consequently influencing spontaneous regression.

Neuroblastoma is especially prevalent within the first year of life, often manifesting outside the cranium, such as in the adrenal medulla. The tumors' nature can vary greatly, with classifications ranging from benign to highly aggressive. The latter can require high modality treatments, while the former has shown indications of spontaneous regression linked to apoptosis. The diversity in tumor environments, particularly in oxygenation levels, further complicates understanding the disease. For instance, hypoxia has been associated both with adverse outcomes and increased apoptosis in NB. With these diverse findings, a pressing question emerges: what instigates apoptosis and could a series of molecular events in NB lead to spontaneous regression?

Central to this investigation is the interaction between TCTP and the NUMB protein, potentially activating the Notch 1 receptor. TCTP's role in tumor reversion has been highlighted in multiple cancers, and its involvement in apoptotic mechanisms has been previously identified. Intriguingly, there's a dynamic between hypoxia and TCTP levels, which can influence its anti-apoptotic functions. NUMB, a known tumor suppressor, interacts with the Notch signaling pathway, often opposing the actions of Notch, an oncogene. Understanding how TCTP, NUMB, and Notch 1 interact can shed light on their collective role in apoptosis, especially in variable environments.

Considering the substantial variability in neuroblastoma, understanding these molecular interactions can provide valuable insights into its treatment. TCTP, which has demonstrated utility in other cancers, offers a promising avenue for exploration in NB. This research hopes to decode whether TCTP's interaction with the Notch signaling pathway can initiate a cascade leading to apoptosis and, ultimately, spontaneous regression.

In another dimension, Oral Lichen Planus (OLP) is a chronic inflammatory condition of the mouth's mucosal surfaces, commonly co-diagnosed with xerostomia. Affecting up to 2% of the population, treatments primarily focus on

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managing inflammation, requiring ongoing care. If unchecked, OLP can evolve into precancerous lesions and even oral cancer. Recent studies have indicated tctP as a vital biomarker for OLP, with its unregulated expression being a marker for poor prognosis in OLP patients progressing to oral cancer. Decoding the genetic mechanisms around the TCTP pathway could unveil therapeutic targets for OLP, paving the way for more targeted treatments, potentially leveraging approaches like siRNA to modulate genes associated with the disease, ultimately aiding in oral cancer prevention.

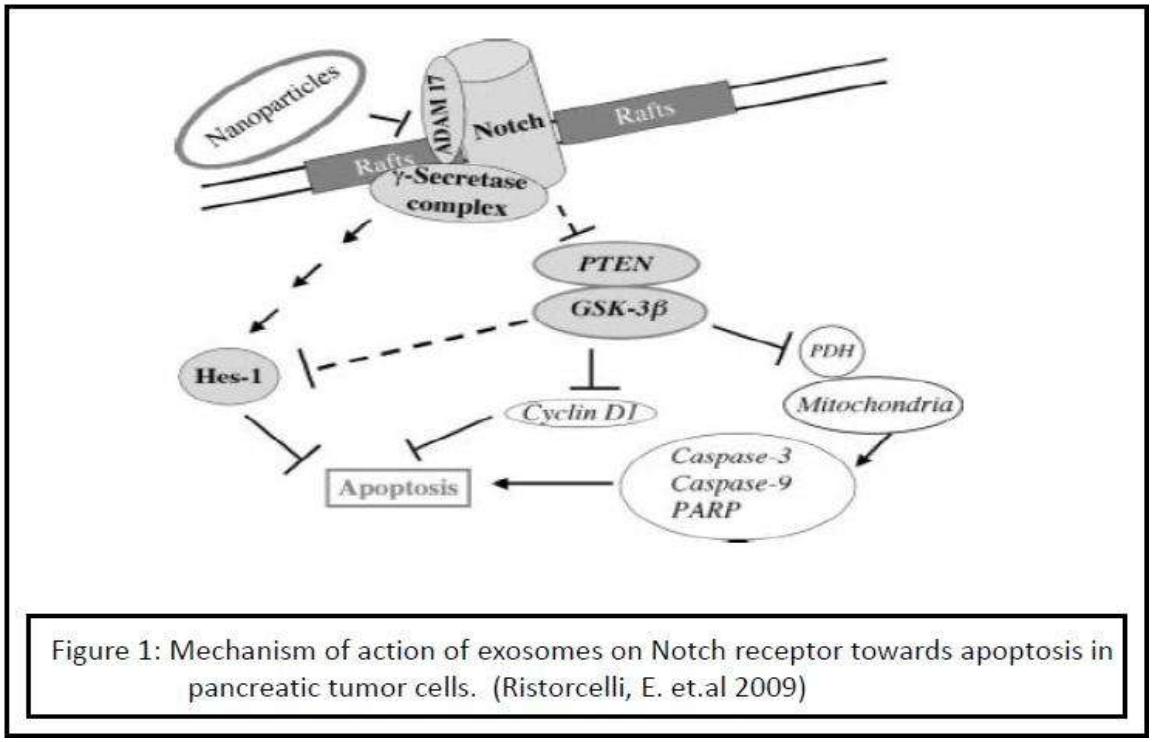

Figure 1: Mechanism of action of exosomes on Notch receptor towards apoptosis in pancreatic tumor cells. (Ristorcelli, E. et.al 2009)

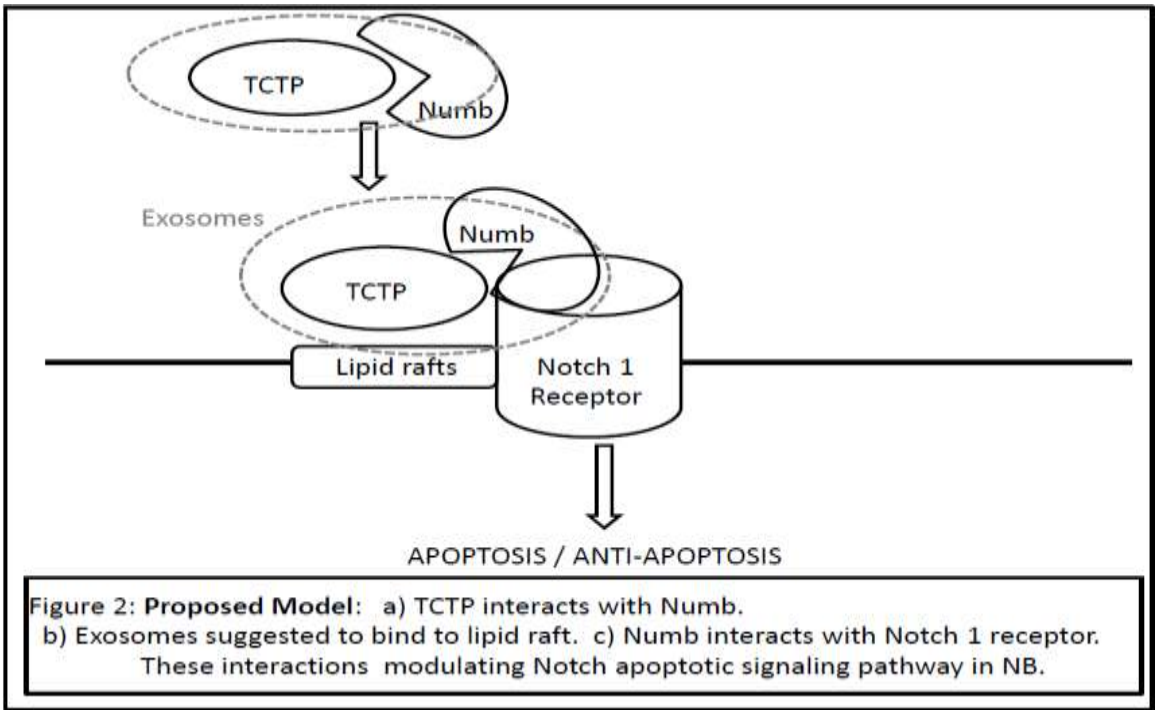

Figure 2: **Proposed Model**: a) TCTP interacts with Numb. b) Exosomes suggested to bind to lipid raft. c) Numb interacts with Notch 1 receptor. These interactions modulating Notch apoptotic signaling pathway in NB.

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IV. RESEARCH DESIGN AND METHODS

SPECIFIC AIM 1

Hypothesis: Explore whether TCTP-bearing exosomes interact with NUMB or Notch 1 receptor in neuroblastoma cells.

Rationale: TCTP is secreted from NB cells and has an extracellular anti-apoptotic effect on vascular smooth muscle cells. Notch 1 receptors are localized in lipid raft domains and lipid-rich exosomes might target these domains. TCTP has been suggested to interact with NUMB protein. This investigation aims to determine if TCTP-bearing exosomes interact with NUMB or Notch 1 receptor on NB cell membranes.

Experimental Method:

1. Cell culture: The neuroblastoma cell lines IMR-32, SK-N-DZ, and SK-N-BE(2) will be maintained under specific conditions.
2. Differential Centrifugation: Exosomes from the NB cell lines will be isolated and prepared for immunoblotting.
3. Immunoblotting: To confirm TCTP presence and interactions, specific antibodies will be used.
4. Coimmunoprecipitation: This will examine the interaction between TCTP and NUMB protein.

Result and Alternate Approaches:

Based on previous findings, TCTP presence without cytoplasmic contamination is anticipated. The interaction between TCTP and NUMB protein, as well as TCTP's association with Notch 1 receptor, will be observed. Statistical analysis will be employed to quantify results, and alternative methods such as confocal microscopy could be employed to further confirm findings.

SPECIFIC AIM 2

Hypothesis: Determine the functional impact of TCTP, Numb, and/or Notch 1 receptor on neuroblastoma cells.

Rationale: TCTP is a protein involved in tumor activity and has an anti-apoptotic effect in several cancer types. Notch 1 receptor influences apoptosis in various cells, and its role in NB could be significant. With NUMB protein inhibiting Notch signaling, it's pertinent to understand if TCTP and/or NUMB's interaction with Notch 1 receptor affects neuroblastoma cell apoptosis.

Experimental Method:

1. NB cell lines IMR-32, SK-N-DZ, and SK-N-BE(2) will be treated and analyzed using immunoblotting and fluorescence microscopy.
2. siRNA method: Different siRNA reagents will be used to transfect the cell lines, with outcomes analyzed through various methods.
3. Immunoblotting: Specific antibodies will identify proteins of interest.
4. Fluorescence microscopy: To determine cell health and apoptotic responses.
5. Recombinant TCTP: Various doses will be added to NB cell lines to discern its effects.

V. RESEARCH DESIGN AND METHODS

Specific Aim 1: Investigate the differential expression between healthy and disease tissues from tooth extractions to identify siRNA targets for OLP intervention.

Hypothesis: Some genes will show differential regulation in diseased tissues compared to healthy tissues.

Rationale: Past research has indicated differential gene expression in patients with oral lichen planus. Given that TCTP plays a role in several cancer types, examining its role in this context might provide valuable insights.

VI. RESULT AND ALTERNATE APPROACHES

Basic Work Principle:

The Agilent‘s SurePrint G3 Human gene expression v3 microarray integrates long non-coding RNA (lncRNA) probes encompassing the entire LNCipedia 2.1 database with updated mRNA probes. The method uses cyanine 3- and cyanine 5-labeled targets to measure gene expression variances between experimental and control samples.

Sample Input, Processing and Data Analysis:

* **Sample Input Type:** Total RNA
* **Sample Input Range:** 10ng-200ng

VII. METHODOLOGY:

1. **Total RNA Extraction:** This will be done from tissue using the RNeasy Mini Kit (Qiagen).
2. **Storage:** The purified RNA will be stored between −80 to −65°C in RNAse-free water.
3. **Quantification, Purity, and Integrity of RNA:** RNA concentration will be gauged with the Qubit 3 Flurometer, while its purity and integrity will be determined by NanoDrop2000.

Two-Color Microarray-Based Gene Expression Analysis:

* Labeling Procedure: Experimental RNA is labeled with Cyanine 5 and Control RNA with Cyanine 3.

* **Steps:**
1. Use Template Total RNA (10-100 ng) with RNA Spike in.
2. Synthesize cDNA using the Reverse Transcriptase Enzyme.
3. Synthesize and label cRNA using either Cyanine 3-CTP or Cyanine-5 CTP with T7 RNA polymerase.
4. Purify the labeled, amplified cRNA.
5. Quantify labeled cRNA using NanoDrop.
6. Pool experimental sample with its corresponding control sample.
7. Fragment pooled, labeled cRNA at 60°C for 30 minutes.
8. Load on the Array slide and hybridize for 17 hours at 65ºC.
9. Wash the Array Slide with buffer.
10. Scan with the Agilent Sure Scan Microarray Scanner.
11. Check the image quality and QC files for each sample using Feature Extraction.

* Amplification typically achieves at least a 100-fold increase from total RNA to cRNA with this kit.
* Data Analysis will employ Gene Spring GX 9.0 software.

VIII. EXPECTATIONS:

In the study, I anticipate confirming TCTP's presence in various media using immunoblotting. I predict increased chromatin condensation in cells with siRNA TCTP pretreatment and in NB cell lines with added media. However, identical apoptosis levels should be present in exosome-free media for both siRNA TCTP and control. A gradient decrease in apoptosis is expected when adding recombinant TCTP to NB cells from the siRNA TCTP group versus the control group.

By inhibiting the Notch 1 receptor, I predict a reduction in apoptosis in NB cell lines and expect further investigations on the influence of TCTP and/or NUMB knockdown on the Notch signaling pathway. I forecast an anti-apoptotic effect when adding Recombinant TCTP to NB cells. Another approach could be using TCTP or NUMB protein inhibitors to understand their effects on the Notch 1 receptor in modulating the Notch apoptotic signaling pathway.

Lastly, for the gene expression analysis, I expect to identify a 3-4 fold change in the TCTP gene expression between healthy and diseased tissues. TCTP might be more expressive in patients with a poor prognosis. Additionally, other potential biomarker genes could be detected, possibly relating to poor prognosis in OLP patients.

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