Dynamin-1 Is A Potential Mediator In Cancer-Related Cognitive Impairment: A Validation Study

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INTRODUCTION

- A protein found in the brain, ***dynamin-1 (DNM1)***, plays a pivotal role in memory formation, and lower DNM1 concentrations have been observed in post-mortem brains of dementia patients[1,2]. As memory impairment is an important sign of cancer-related cognitive impairment (CRCI)[3], ***it is hypothesized that downregulation in DNM1 may be observed in those experiencing CRCI.***
- Our exploratory study reported a greater downregulation in DNM1 levels when comparing plasma extracellular vesicle (EV) proteomes of CRCI breast cancer survivors against those without CRCI. However, the study utilized pooled participant samples and was thus unable to adjust for variables that could confound the DNM1-CRCI relationship[4].
- This follow-up study leverages on a prospective cohort of adolescent and young adult (AYA, 15-39 years old at cancer diagnosis) cancer survivors and uses individual participant samples to validate our preliminary findings that DNM1 downregulation during cancer treatment underlies CRCI pathophysiology[4,5].

RESEARCH QUESTION

- ***How do changes in DNM1 levels during cancer treatment differ between AYAC patients with and without CRCI?***

METHODS

- **Study design:** Prospective cohort study conducted at the National University of Singapore, National Cancer Center Singapore and KK Women's and Children's Hospital between June 2018 and June 2022. The study protocol received ethics approval from SingHealth Institutional Review Board (CIRB 2017/3139).
- **Participants:** Two groups of participants were recruited for the study. AYA cancer patients (AYAC) and age-matched non-cancer community controls (NC).
- **Study timepoints:** AYAC performed the study procedures at baseline (T1), approximately 3 (T2), and 6 (T3) months after baseline. NC will be followed up at baseline (T1) and approximately 6 (T3) months after baseline (Figure 1).

METHODS (contd.)

- **Procedures:** The following study procedures were conducted at each timepoint (T1, T2, T3, Figure 1).
  1. Self-reported assessment of cognitive function ***(FACT-Cog)***, psychological distress ***(Rotterdam Symptom Checklist)*** and fatigue ***(Multidimensional Fatigue Symptom Inventory—Short Form)***;
  2. Blood draws for plasma collection (1,069 *x g* for 10 minutes), stored at -80°C until ***DNM1*** quantification.

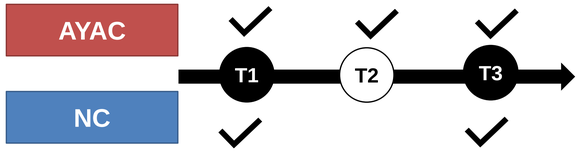

Figure 1: Study timepoints, denoting as T1, T2 and T3 for baseline, approximately 3 and 6 months after baseline, respectively.

- ***DNM1 quantification:*** EVs were isolated from plasma samples via differential ultracentrifugation[4,6]. DNM1 levels were measured with a commercially available ELISA kit (ABclonal RK01275, USA) and then normalized by EV concentration (dividing by EV marker FLOT1 levels). DNM1 levels are presented as pg/ng (picogram of DNM1 per nanogram of FLOT1).
- ***Definition of cognitive impairment (CRCI or CI):*** Clinically significant 10.6-point reduction in global FACT-Cog score from baseline[7].
- **Statistical analysis:** DNM1 levels between baseline (T1) and follow-up (T2 or T3) timepoints were first evaluated among AYAC after stratification by CRCI status (yes/no) with paired t-test or Wilcoxon signed-rank test. Change/day in DNM1 were estimated for each of the four subgroups (AYAC +/- CRCI, NC +/- CI) with generalized estimating equations (GEE). Adjusted confounders include baseline DNM1 levels, cancer status, age, gender, ethnicity, marital status, education years, as well as change in psychological distress and fatigue levels.

RESULTS

(A) Participant characteristics

- Samples from ***30 AYAC*** and ***56 NC*** participants were available for analysis. AYAC were mostly diagnosed with breast (33%) and head/neck (20%) cancers (Table 1).
- At T2, there were 7 (23%) AYAC with CI. At T3, there were 8 (27%) AYAC and 10 (18%) NC participants with CI.

Table 1: Participant characteristics.

Characteristics	AYAC (N=30)	NC (N=56)	*P*
Age in years, median (IQR)	35 (30, 37)	33 (27, 35)	0.073
Female, n (%)	20 (67%)	40 (71%)	0.650
Ethnicity, n (%)			0.280
Chinese	23 (77%)	42 (75%)	
Malay	3 (10%)	2 (4%)	
Indian	1 (3%)	8 (14%)	
Others	4 (7%)	4 (7%)	
Married, n (%)	16 (53%)	24 (43%)	0.374
Years of education, median (IQR)	16 (13, 17)	17 (15, 18)	**0.035**

(B) Changes in DNM1

- **Univariate analysis:**
  - Statistically significant increase in DNM1 levels in EVs were found among AYAC without CRCI at T2 (p=0.007, Figure 2A) but not at T3 (p>0.05, Figure 2B).
- **Multivariate analysis:**
  - ***Utilizing GEE adjusted for confounders, we found statistically significant reductions in DNM1 levels among AYAC with CRCI (β=-0.15, 95% CI=-0.29 to -0.01, p=0.033).***
  - No significant changes were found in other subgroups of participants (AYAC without CRCI, NC +/- CI, p>0.05).

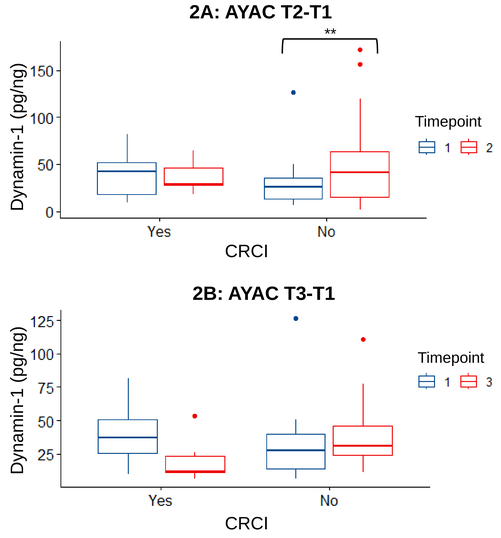

Figure 2: Dynamin-1 levels among AYAC participants stratified by CRCI status at (2A) T2 and (2B) T3. *(**p < 0.01)*

DISCUSSION

- Our findings are congruent with our previous study[4] and validated that DNM1 downregulation is observed in cancer patients who experienced self-perceived CRCI while receiving cancer treatment. This is also likely specific to CRCI given that young-adult NC who reported poorer cognition were not found with differentially expressed DNM1 compared to NC counterparts with better cognitive function.
- Because DNM1 plays a crucial role in ensuring effective neurotransmission by facilitating synaptic vesicle recycling via endocytosis, depletion in DNM1 levels may reflect reduced neuronal activity dependent on synaptic vesicles[8,9]. Preclinical studies may confirm the importance of this mechanism in CRCI pathophysiology.
- This study is innovative for being the first to quantify DNM1 activity with peripheral blood, and for adding to existing literature regarding the potential of EVs as biomarkers to research on cancer-related complications. DNM1 has great potential to serve as a therapeutic target for CRCI.

CONCLUSIONS

- We have provided additional clinical evidence that DNM1-associated neuronal activity may be reduced among cancer survivors experiencing CRCI. Future studies may confirm our proposed mechanism in preclinical models of CRCI and explore the use of neuronal-derived plasma EVs for a more accurate representation of DNM1 activity in the neurons[10].

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Acknowledgements

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