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TITLE: Identification of Neoantigens from lncRNA-Encoded Micropeptides in Kidney Cancer

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13. SUPPLEMENTARY NOTES

Rationale: Kidney cancer is the eighth most common malignancy in the United States, and the 5 year survival rate for the metastatic kidney cancer is only about 12%. Thus, there is an urgent need for better treatment of kidney cancer. Checkpoint blockade immunotherapy represents a novel therapy and has been successfully to treat various types of cancers such as melanoma and lung cancer. Neoantigens, generated from tumor mutational burden (TMB) play a critical role in immunotherapy. Common approaches for identification of neoantigens are through sophisticated processes such as deep sequencing of tumor vs normal, bioninformatics analysis and experimental validation. To date, all neoantigens are derived from mRNAs. In this application, we propose to identify such neoantigens from peptides encoded by long non-coding RNAs (lncRNAs).
Objectives: Since lncRNAs could encode a very large number of micropeptides that may play a role in normal cell functions or in cancer, we hypothesize that as a large source of peptides encoded from lncRNAs, some of them can serve as neoantigens which could be novel immunotherapeutic targets. Thus, the overall objective is to address two critical issues that can greatly influence the identification of bona fide immunogenic neoantigens: 1) the identification of candidate neoantigens, and 2) the evaluation of their immunogenicity.
Methods: We will first generate a virtual peptide library derived from lncRNA-encoded small open reading frames (sORFs). The micropeptides with the binding affinity of <500 nmole/L will be used for further analysis. Second, to further narrow down the number of potential micripeptides, we will determine whether the identified neoantigen candidates are conserved in mouse or other species because conserved sequences indicate potential biological functions, and evolutionary conservation may serve as another predictor in the analysis of functions of the micropeptides. Finally, we will select top five micropeptides with the highest binding affinity to HLA-A02:01 and express cDNAs corresponding to these micropeptides in kidney cancer cells.

15. SUBJECT TERMS
Kidney Cancer, lncRNA, Micropeptides, immunotherapy, HLA complex

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Neoantigens, generated from tumor mutational burden (TMB) play a critical role in immunotherapy. Common approaches for identification of neoantigens are through sophisticated processes such as deep sequencing of tumor vs normal, bioninformatics analysis and experimental validation. To date, all neoantigens are derived from mRNAs. In this application, we propose to identify such neoantigens from peptides encoded by long non-coding RNAs (lncRNAs).

KEYWORDS:

Kidney Cancer, lncRNA, Micropeptides, immunotherapy, HLA complex

ACCOMPLISHMENTS:

What were the major goals of the project?

Since lncRNAs could encode a very large number of micropeptides that may play a role in normal cell functions or in cancer, we hypothesize that as a large source of peptides encoded from lncRNAs, some of them can serve as neoantigens which could be novel immunotherapeutic targets. Thus, the overall objective is to address two critical issues that can greatly influence the identification of bona fide immunogenic neoantigens: 1) the identification of candidate neoantigens, and 2) the evaluation of their immunogenicity.
We will first generate a virtual peptide library derived from lncRNA-encoded small open reading frames (sORFs). The micropeptides with the binding affinity of <500 nmole/L will be used for further analysis. Second, to further narrow down the number of potential micripeptides, we will determine whether the identified neoantigen candidates are conserved in mouse or other species because conserved sequences indicate potential biological functions, and evolutionary conservation may serve as another predictor in the analysis of functions of the micropeptides. Finally, we will select top five micropeptides with the highest binding affinity to HLA-A02:01 and express cDNAs corresponding to these micropeptides in kidney cancer cells.

What was accomplished under these goals?

Major Task 1: To generate a virtual peptide library

Subtask 1, We will compile all those human lncRNA-encoded peptides which have been experimentally detected by mass spectrometry analysis from literature.

We have compiled 134,124 human lncRNA-encoded peptides which have been experimentally detected by mass spectrometry analysis from literature as shown in Table 1.

Major Task 2: To predict the binding affinity of micropeptides to HLA-A02:01

Subtask 1: We will use two programs OptiType (https://github.com/FRED-2/OptiType) and NetMHCpan (http://www.cbs.dtu.dk/services/NetMHCpan/) to predict the binding affinity of each micropeptide.

We have predicted the binding affinity of each of 134,124 peptides as shown in Table1.

Table 1 Library of peptides encoded by lncRNA and affinity score

	Peptide_ID	MHC I					MHC II					Combined
		SB	WB	LA_Freq	LA_Freq	I_Score	SB	WB	LA_Freq	LA_Freq	II_Score	Score
1	P_60890	2126	4285	1.11	1.11	11801.35	278	2680	0.661	1.315	1358.65	11937.22
2	P_60896	1958	4447	0.95	1.06	10313.26	9	185	0.055	0.217	13.87	10314.65
3	P_125605	606	1359	1.05	1.11	3416.41	13358	51373	1.370	1.379	41908.57	7607.26
4	P_125633	529	1205	1.05	1.11	3002.98	14279	48252	1.379	1.379	41865.73	7189.55
5	P_125746	335	752	1.00	1.11	1834.95	17881	61637	1.319	1.379	51905.31	7025.48
6	P_4756	635	1781	1.11	1.11	4079.51	8064	21790	1.379	1.379	21133.95	6192.90
7	P_4773	635	1781	1.11	1.11	4079.51	8064	21790	1.379	1.379	21133.95	6192.90
8	P_125781	310	697	0.98	1.11	1685.64	13477	47661	1.319	1.379	39675.08	5653.14
9	P_94671	809	2146	1.11	1.11	5061.20	1163	5893	0.819	1.328	3560.33	5417.23
10	P_102872	490	1348	0.92	1.07	2794.66	6774	20416	1.276	1.379	18024.68	4597.12
.	.											
.	.											
.	.											
28166	P_116966	102	243	0.79	1.04	495.044	81	346	0.113	0.354	49.91	500.035
28167	P_116858	102	243	0.79	1.04	495.044	81	346	0.113	0.354	49.91	500.035
28168	P_78576	78	298	0.66	1.11	483.570	33	651	0.127	0.739	164.52	500.022
28169	P_125554	86	206	0.90	0.99	437.064	134	1368	0.254	1.305	629.24	499.988
28170	P_53133	50	180	0.39	0.96	231.358	465	5163	0.750	1.358	2686.15	499.974
28171	P_53181	50	180	0.39	0.96	231.358	465	5163	0.750	1.358	2686.15	499.974
.	.											
.	.											
.	.											
134117	P_52440	0	2	0.00	0.01	0.025	0	0	0	0	0.00	0.03
134118	P_126631	0	2	0.00	0.01	0.020	0	0	0	0	0.00	0.02
134119	P_39760	0	1	0.00	0.00	0.001	0	6	0.000	0.074	0.15	0.02
134120	P_92074	0	1	0.00	0.01	0.010	0	54	0.000	0.001	0.01	0.01
134121	P_29816	0	1	0.00	0.01	0.010	0	8	0.000	0.001	0.00	0.01
134122	P_125634	0	1	0.00	0.01	0.010	0	2	0.000	0.000	0.00	0.01
134123	P_112001	0	1	0.00	0.01	0.010	0	0	0	0	0.00	0.01
134124	P_133139	0	1	0.00	0.01	0.006	0	0	0	0	0.00	0.01

Subtask 2: Those micropeptides that have the binding affinity of <500 nmole/L will be subject to further analysis.

We are working on further analysis of micropeptides that have the binding affinity of <500 nmole/L. for their length of peptide, number of fragments within peptide, sequence of each fragment, and affinities and the number of epitopes with 8-11 aa within each peptide.

Major Task 3: To determine the sequence conservation of neoantigen candidates (Not initiated)

Subtask 1: We will use programs such as Liftover (https://genome.ucsc.edu/cgi-bin/hgLiftOver to identify orthologous genomic locations of human lncRNA ORFs in mouse, and obtain possible ORFs flanking these regions.

Major Task 4: To evaluate their immunogenicity (Not initiated)

Subtask 1: We will select top five micropeptides with the highest binding affinity to HLA-A02:01 and express cDNAs corresponding to these micropeptides in kidney cancer cells (Caki-1).
Subtask 2: To determine which fragments of micropeptides are processed and bound to HLA complex, we will precipitate HLA-peptide complex using the capture antibody against soluble HLA-A coupled to agarose beads,
Subtask 3: Identify amino sequences of recovered peptides by MS analysis.
Subtask 4: We will generate neoepitope-specific T-cell clones and neoepitope-specific T-cells by TCR gene transduction.
Subtask 5: We will then perform in vitro peptide binding assay and cytotoxicity assay.

Nothing to Report

How were the results disseminated to communities of interest?

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?

lab personnel has been recruited and starts work in January 2, 2024.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to report.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

Nothing to report.

Changes in approach and reasons for change

Actual or anticipated problems or delays and actions or plans to resolve them

Due to pandemic, it was unable to recruit lab personnel on time. Now the recruitment has completed.

Changes that had a significant impact on expenditures

No change.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

No changes.

Significant changes in use or care of vertebrate animals

No changes.

Significant changes in use of biohazards and/or select agents

No changes.

6. PRODUCTS:

Nothing to report.

- **Publications, conference papers, and presentations**

  Journal publications.

Nothing to report.

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers and presentations.

Nothing to report.

- **Website(s) or other Internet site(s)**

Nothing to report.

- **Technologies or techniques**

Nothing to report.

- **Inventions, patent applications, and/or licenses**

-

- Nothing to report.

Other Products

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

No change.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: ***N/A***

QUAD CHARTS: ***N/A***

9. APPENDICES: *N/A*