Identification of neoantigens as potential vaccines in hepatocellular carcinoma

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Introduction / Aim:

Neoantigens (neoAgs), new immunogenic sequences arising because of tumor mutations[1], have been associated with response to immunotherapy and are considered potential targets for vaccination[2]. Hepatocellular carcinoma (HCC) is a moderately mutated tumor[3], where the neoAg repertoire has not been characterized.

The aim of this study is identify neoAgs in HCC patients, testing their immunogenicity for future use in therapeutic vaccination.

Methods:

Whole exome sequencing and RNAseq were performed in a cohort of 14 HCC patients (shown in Table 1) submitted to surgery or liver transplant. To identify mutations, single nucleotide variants (SNV) originating non-synonymous changes not reported in data bases that were confirmed at the RNA level were analyzed. To identify potential neoAgs, in silico HLA binding algorithms (NetMHCpan 4.0 and NetMHCIIpan 3.2) were used.

For in vitro HLA-A2.01 binding assays T2 cells were cultured with neoAg peptides at 100 µM. 24 hours later expression of HLA-A2.01 was determined by flow cytometry. Binding was expressed as fluorescence index (FI): (FI with peptide – FI without peptide)/FI without peptide. FI > 0.5 was considered positive.

For HLA-DRB1 binding determination, HOM2 cells were cultured with neoAg peptides at 100 µM in the presence of the control biotinylated peptide (HA306–320). 24 hours later binding of neoAg peptides was determined by flow cytometry as inhibition of the control peptide and was expressed as binding score (BS): BS= 100× (signal inhibition with peptide tested)/(signal inhibition with unbiotinylated HA306–320). Peptides with BS > 0.5 were considered positive.

For in vivo assays HHD transgenic mice expressing the human HLA-A2.1 and HLA-DRB1 alleles, (n = 3/group) were vaccinated with 100 nmoles neoAg + 50 µg Poly(I:C) + 50 µg antiCD40. For class I peptide testing mice received one immunization and for class II, they received two weekly immunizations. Seven days after the last vaccination splenocytes were stimulated with mutated peptides to determine by ELISPOT neoAg-specific responses. NeoAgs inducing more than 100 IFN-γ SFC per 8x105 cells were considered immunogenic. In some cases splenocytes were stimulated with cells transduced with plasmids encoding the neoAgs. Peptide stimulated T cells were also analyzed by flow cytometry measuring expression of IFN-γ, TNF-α, and CD107.

Conclusion:

These results show that mutations arising in HCC tumors may generate neoAgs recognized by CD8 and CD4 T cells, with potential applicability for therapeutic vaccination.

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References:

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2. Yarchoan, M., Hopkins A., Jaffee E. Tumor Mutational Burden and Response Rate to PD-1 Inhibition. N Engl J Med 377, 2500-2501 (2017). https://doi.org/10.1056/NEJMc1713444.
3. Alexandrov, L., Nik-Zainal, S., Wedge, D. et al. Signatures of mutational processes in human cancer. Nature 500, 415–421 (2013). https://doi.org/10.1038/nature12477.

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Results:

Table 1. Characteristics of HCC patients included in the study.

Patient ID	Tx	Age	Cirrhosis	Child-Pugh	HBV	HCV	EtOH	NASH	Size *(cm)*	AFP	BCLC	Previous Tx
10584	RE	58	Yes	A	Yes	Yes	No	NO	4	2.7	A	No
10594	RE	46	No	-	No	Yes	No	No	3.5	8.7	A	No
10615	RE	70	No	-	No	No	Yes	Yes	11	21.4	A	RE
10619	TR	50	Yes	C	No	Yes	Yes	No	2 (2 lesions)	4.8	D	No
10622	RE	71	Yes	A	No	No	No	No	1.2	-	A	RE
10627	RE	77	Yes	A	No	No	Yes	Yes	1.3	-	A	RE
10628	TR	59	Yes	A	No	Yes	No	No	0,5	1	A	RE
10632	RE	59	Yes	A	No	Yes	No	No	2	3.4	A	RE, SOR, ICI
10634	TR	67	Yes	A	No	No	Yes	Yes	2.6 (2 lesions)	2	A	No
10635	TR	67	Yes	-	No	No	Yes	No	3 (2 lesions)	4	A	No
HLA063	RE	83	Yes	-	No	Yes	No	No	2	12	A	No
HLA066	RE	71	No	A5	No	Yes	No	No	1.2	8	A	No
HLA078	RE	68	Yes	A6	No	Yes	No	No	10	4.5	A	No
HLA069	RE	69	No	A5	Yes	No	No	No	3	5.2	A	No

Tx, treatment; RE, resection; TR, transplant; SOR, sorafenib; and ICI, immune checkpoint inhibitor.

Table 2. NeoAg selection from WES and RNAseq data.

		10584	10594	10615	10619	10622	10627	10628	10632	10634	10635	HLA063	HLA066	HLA069	HLA078	Median
Mutations	Total WES	1217	1115	4018	1112	984	1083	1218	1204	1103	1308	5185	2862	4318	5118	**1217,5**
	Missense	350	267	1247	276	270	328	296	353	274	380	1409	735	1061	1560	**351,5**
	Not reported	263	196	273	196	190	235	200	243	205	296	1093	544	819	718	**253**
	TUM RNA	16	19	41	21	26	14	10	33	-	30	101	40	60	38	**30**
	NT RNA	15	18	38	21	23	13	9	32	-	30	94	34	42	32	**30**
Predicted Peptides	HLA-I Binding	17	8	10	12	13	7	6	14	-	17	-	-	-	-	**10**
	HLA-II Binding	4	4	6	7	4	1	1	5	-	5	-	-	-	-	**4**
	Total	11	12	16	19	17	8	7	19	-	22	-	-	-	-	**16**
HLA-A2.01 Binding		3	4	5	5	-	-	-	-	-	-	6	1	-	2	**3,5**
HLA-DRB1 Binding		-	-	-	-	-	-	-	4	-	4	-	-	1	-	**4**

WES, whole exome sequencing; TUM, tumor; NT, non-tumor.

1. Experimental confirmation of binding to HLA-A2.01 of predicted neoAg peptides.

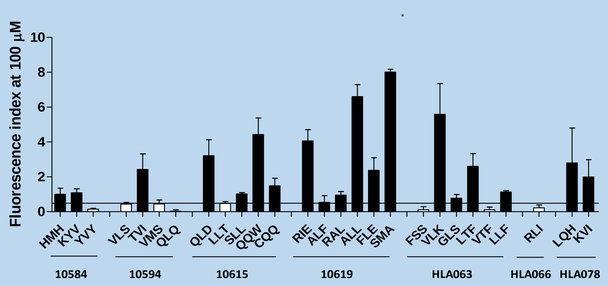


***Figure 1**: In vitro assays with T2 cells were performed to determine binding to HLA-A2.01*

2. *In vivo* immunogenicity in HHD mice of HLA-A2.01-predicted binders.

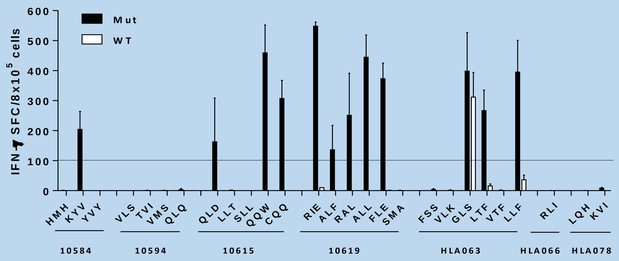
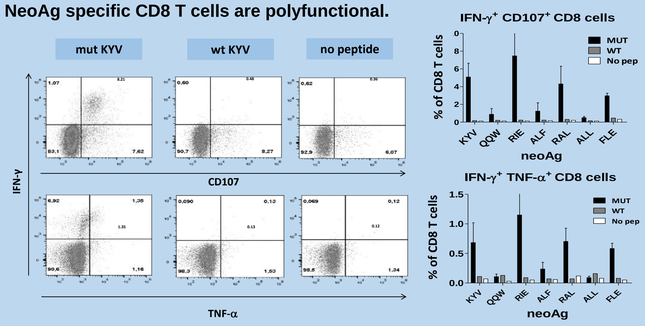


***Figure 2**: Splenocytes from immunized HHD mice were stimulated with the mutated or WT version.*

3. NeoAg specific CD8 T cells are polyfunctional.



4. Binding to HLA-DRB1.01 and immunogenicity of predicted neoAg peptides.

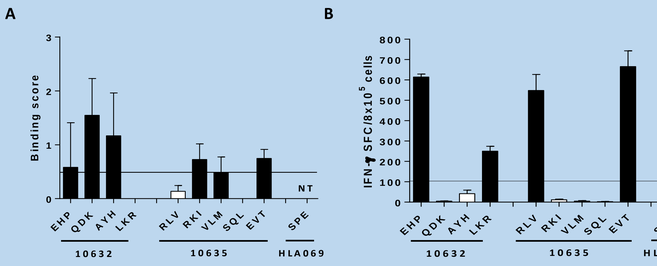


***Figure 4**: A) vitro assays with HOM2 cells were performed to determine binding to HLA-DRB1. B) Splenocytes from immunized HHD mice were stimulated with the neoAg. NT, not tested.*

5. NeoAg peptide-activated T cells recognize epitopes after Ag processing.

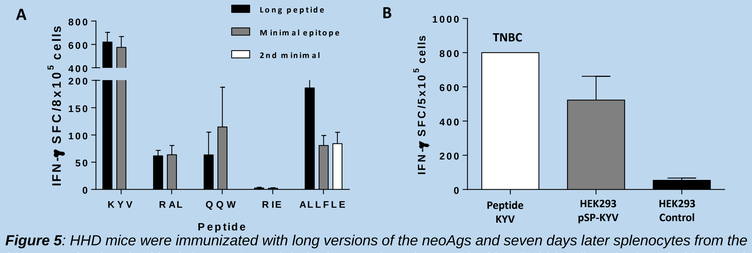


***Figure 5**: HHD mice were immunizated with long versions of the neoAgs and seven days later splenocytes from the mice were stimulated with A) the neoAg and their elongated version, or B) with HEK293 cells transduced with plasmid expressing the neoAg. TNBC: Too numerous to be counted.*

6. Co-immunization of CD8 and CD4 neoAgs result in stronger CD8 T cell responses.

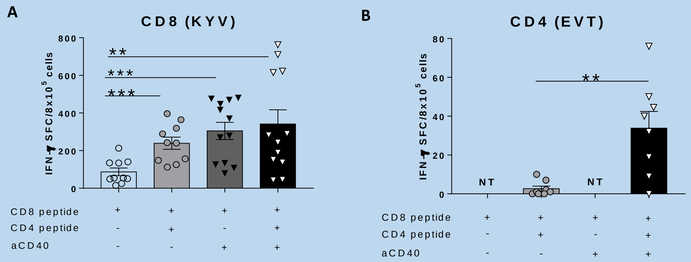


***Figure 6**: Splenocytes from immunized HHD mice were stimulated with the CD8 or CD4 epitopes. NT, not tested.*