Common Cancer Neo Antigens from the Frame Shifted Transcripts

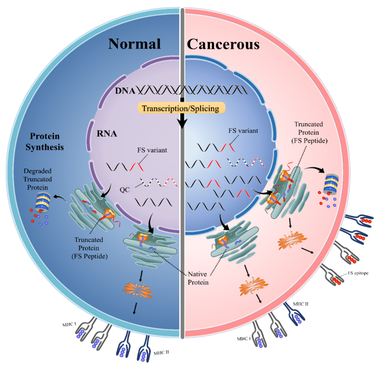
Luhui Shen, Ji'an Zhang, Hojoon Lee, Milene Tavares Batista, Stephen Albert Johnston*

Center for Innovations in Medicine, The Biodesign Institute, Arizona State University, Tempe, Arizona, 85287

Background

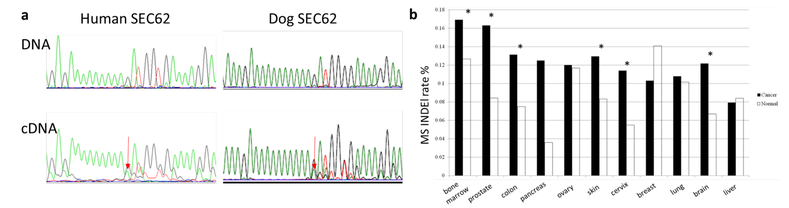
The recent breakthrough of cancer immunotherapy with immune checkpoint inhibitors (CPI) not only showed dramatic efficacy in multiple cancer treatments, but also suggested that tumor neo-antigens are the targets of the anti-tumor immune responses that were released by the CPI treatments. The most recently studies of neo-antigen based personalized human melanoma cancer vaccines (PVC) further support this hypothesis by showing efficacy in preventing cancer metastasis and recurrence. However, DNA sequencing of tumors indicates that almost all neo-antigens are personal. In contrast, we propose a model of cancer that suggests there is a source of common cancer neo-antigens encoded by frame shift transcripts. It is based on the concept that the initiation event of a potentially cancerous cell will destabilize basic cellular processes like transcription and RNA splicing. Insertion or deletion (INDELs) in the transcription across microsatellites (MSs) and mis-splicing of exons will produce frameshift (FS) transcripts, encoding frameshift peptides as highly immunogenic neo-antigens. The increasing expression of the FS peptides along with other aberrant proteins would overload the protein quality control system allowing presentation of the FS epitopes to the immune system. This model predicts that these FS variants will be encoded in the RNA but not DNA, most would be immunogenic and could serve as a vaccine component. Here we have tested each of these predictions using data from mice, dogs and humans. Each of the predictions are supported. This model implies that there may be a simpler approach to developing personal vaccines and that a general therapeutic or preventative vaccine may be possible.

Model for broad frame-shift peptide production

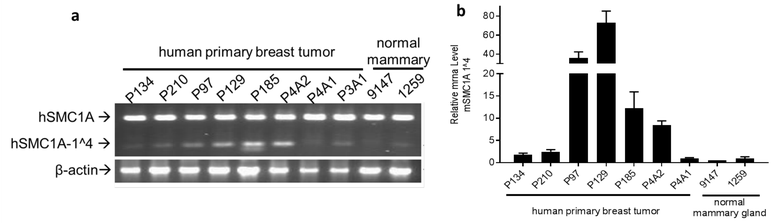


The model for broad, frame-shift peptide production in tumor cells. Normal Cell: Both transcription error and mis-splicing during intron excision. Additionally, the FS transcript with a premature termination may be degraded by Nonsense Mediated Decay (NMD). Aberrant proteins, including those with frameshifts are largely eliminated by the protein quality control system. The net result is that very few frameshift peptides are presented on MHC I/II or not to be presented to the immune system. **Cancer Cell**: All levels of information transfer become more error prone. More errors are made in DNA replication, but only when cells divide. Most DNA mutations are point mutations and encode low or non-immunogenic peptides. Global transcription is increased and is generally less accurate and even more so through MSs producing INDELs. RNA splicing is also far less accurate, creating more frameshift transcripts from each out-of-frame splicing between exons from the same gene and different genes. The substantial increase of the FS transcript from INDELs of MS and mis-splicing overwhelms RNA quality control systems, such NMD. Consequently, more truncated proteins with the FS peptide will be translated. These un-folded proteins, combined with aberrant proteins from other mutations, overwhelms the protein quality control system leading to more frameshift peptides being presented on MHC I/ II and mis-secreted or released from the cancer cell which the immune system can respond to.

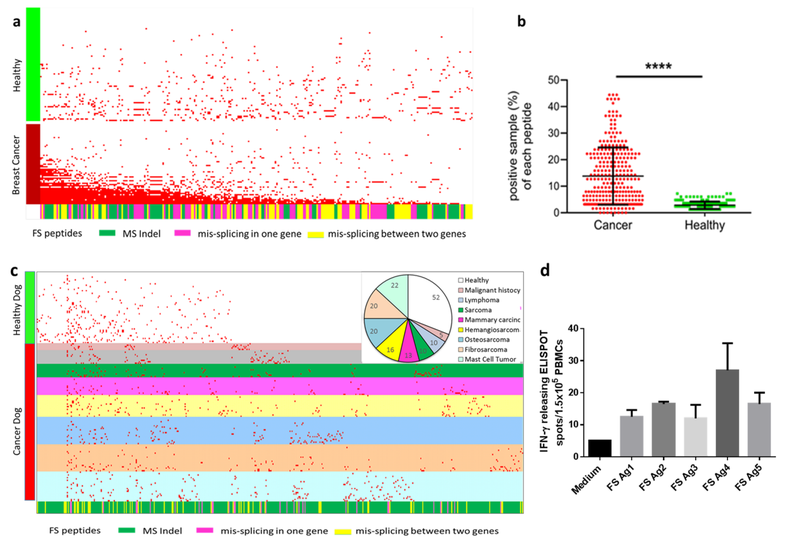
Detection of FS in Human and Dog tumors



FS transcript from INDEL of MS. a. FS only detected in RNA. Example of sequence trace of the MS region in SEC62 dog and human genes in paired DNA/cDNA samples. **b. MS INDEL rate is higher in cancers**. Homopolymer (repeat bp>7) MS Indel rate in EST database between different cancer and normal libraries. * indicate P<0.0001.



Example of FS transcript from mis-splicing. a. End-point RT-PCR analysis of hSMC1A-1^4 expression in human primary breast tumor tissues and normal mammary tissues. **b. The hSMC1A-1^4 is higher in BC**. RT-qPCR analysis of the relative expression level of hSMC1A-1^4 in human primary breast tumor tissues and normal mammary tissues. All values are normalized relative to the expression levels in sample 1259 (set as 1).

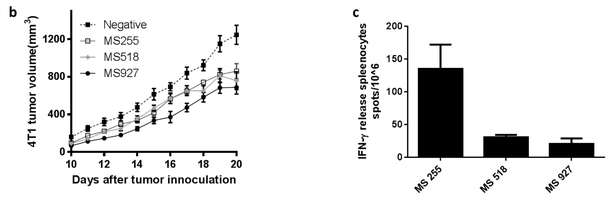


Detecting Immune Response to FS antigen in both human and dog cancer patients. a. Overview of the IgG positive reactivity of each FS peptide in each breast cancer patient (n=63) and non-cancer subjects (n=83) by human FS peptide array. Color coded X axis indicates difference sources of the FS peptide. The positive cut off value of each peptide was calculated as the mean reactivity in non-cancer subjects plus 2 fold SD. **b.** Percentage of positive samples of each peptide in cancer patients and non-cancer subjects. **** , p<0.0001 by two tailed T-test. **c.** Overview of the IgG reactive to the dog FS peptides on the dog FS peptide array with 8 different cancers dogs (n=116) and healthy dogs (n=52). Red spots indicates the median normalized IgG reactive to the FS peptide > 5. **d.** Detecting T cell reactive to the FS antigens with high IgG reactive on the FS array in a dog cancer patient.

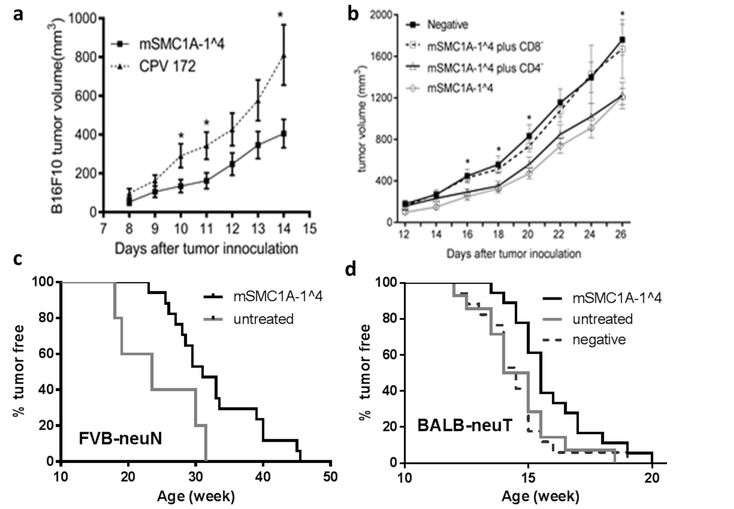
Protection of FS Ag based vaccine in mouse models

a

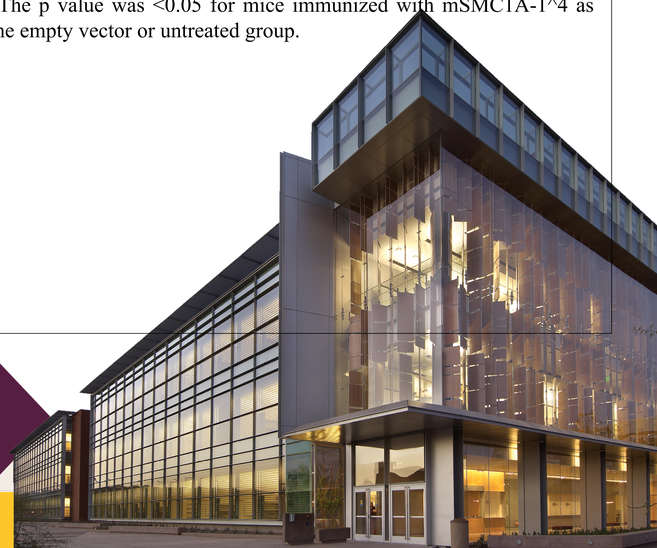
Antigen ID	Access #	MS type	INDEL	Peptide size	peptide sequence (Kd/Ld epitope score>20)
MS927	NM_053009.3	9_A	Del	33	ICMSPPLLWATLQAPETTSAACKASYRPEGLYL
MS255	NM_010086.4	9_A	In	24	YFSCDKRCIKHYAGNKSLLTFSGY
MS518	NM_153511.3	10_A	Del	59	TLCMEVMLRWNTRELGYLYLQLCFLNTHFLHTSQEEKLLTLGRFLTWTSRCGSFVIRPL



Example of protection by any predicted FS Ag. a. Three FS antigens were selected by their MHC I epitope prediction. **b.** Tumor growth curve by three MS FS antigens immunization with 4T1-BALB/c tumor model. Mice (n = 10 per group) were challenged with $5x10^3$ 4T1 cells 2 weeks after the last immunization. **c.** ELISPOT analysis of the immune response by the three MS FS antigen immunization. Data presented here were subtracted the spots in the controls.



Example of broad protection of a FS Ag in different tumor models. a. Tumor growth curve of mSMC1A-1^4 immunization in the B16F10-C57BL6 tumor model compared to the non-protective Cowpox viral antigen. Mice (n = 10) were challenged with 10^5 B16-F10 cells 4 weeks after the immunization. **b**. Tumor growth curve after mSMC1A-1^4 immunization in the 4T1-BALB/c tumor model. Mice (n = 10) were challenged with $5x10^3$ 4T1 cells 2.5 weeks after the last immunization. The CD8 and CD4 T cell depletion started 2 weeks after the last immunization. The negative group in immunized empty vectors and boosted with the KLH protein, Error bars in all mouse growth curve represent SEM, *, p<0.05 and ** , p<0.005 by two tailed t-test. **c.** Tumor-free curve of FVB-neuN female mice (n = 17 for mSMC1A-1^4 and n = 5 for untreated). Mice were genetically immunized at 4-5 weeks of age. The p value = 0.025. c. Tumor-free curve of the BALB-NeuT mice immunized with mSMC1A-1^4 (n = 18), with empty vectors (n = 17), or untreated (n = 14). The p value was <0.05 for mice immunized with mSMC1A-1^4 as compared to either the empty vector or untreated group.



ASU Biodesign Institute
Arizona State University

www.biodesign.asu.edu