

Increased transcription infidelity in cancer cells deregulate innate humoral immunity application to lung and breast cancer diagnosis.

Comparison of m-RNA derived sequences from normal and cancer cells revealed statistically significant increase in single base substitution, insertion and gap in cancer. These events are  $10^3$  more common than somatic mutations and do not correspond to known SNP. Occurrence of these events is strongly conditioned by the composition of affected base as well as that of the 3 bases immediately upstream and the 4 immediately downstream. This 8 base motif corresponds to the segment of DNA melted by PolII during transcription. This led to the hypothesis that transcription infidelity occurred at increasing rate in cancer cells. In silico translation of mRNA with TI event led to prediction of several TI proteins that were identified by mass spectrometry performed on normal and cancer cells. Thus TI is a normal phenomenon contributing to the diversity of the normal human proteome but production of TI proteins increases in cancer cells.

Single base gap is the TI even that most increase ( $> 14$  fold) in cancer cells; thereby causing the production of proteins with altered carboxy-terminal amino-acid sequences. Through mice studies we discovered that TI peptides produced from mRNA with single base gap contain specific epitopes of natural IgG. i.e. immunoglobulin present in immuno-competent mice in absence of known immunogenic challenges. Adoptive transfer of Lewis Lung Carcinoma to syngenic C57 bl6 mice caused first a decrease than increase of IgG directed against specific TI peptides that the mice genome is able to encode but not against TI peptide that the mice genome is unable to encode nor against peptide with canonical amino-acid sequence. This humoral immunogenic response is both T and B cell dependant.

IgG directed against 60 in silico predicted TI peptides were detected in all normal human subjects at levels statistically significantly above those of 3 canonical peptides that remains at background level. The presence of early stage lung cancer causes detectable distortions of this natural IgG network. Probing of these distortions with a panel of TI peptides led in cohort of 131 early stage lung cancer tested versus 130 age and sex matched controls to diagnostic performance of 87 % sensitivity and 98 % specificity. Replication studies using a panel of 24 TI peptides and including 92 lung cancers from 3 independent centres and covering all stages of the disease versus 156 controls that include patients with pathologically proven benign lung mass and patients with active viral and bacterial diseases led to diagnostic performances of  $> 90$  % specificity and specificity  $>98$  %. Using a different panel of TI peptides, we tested a cohort of 140 breast cancer patient versus 157 age matched controls and achieved diagnostic performance of 95 % sensitivity and 99 % specificity. Replication using 20 patients with breast cancer and 40 controls as well as 20 patients with lung cancer showed similar level of performance with one breast cancer patient misclassified and no lung cancer identified by breast cancer test. In a second replication study using 35 breast cancer and 28 patients undergoing surgery for benign breast disease all breast cancers were correctly classified with no benign disease misclassified.

We therefore propose that TI is a normal phenomenon contributing both to the heterogeneity of the normal human proteome and to defining the epitopes specifically recognized by natural IgG. Increased production of TI proteins by cancer cells deregulates innate humoral immunity and probing of these cancer specific deregulations provides novel early stage diagnostic methods. The clinical value of these novel tests is being evaluated in ongoing multi-centric prospective case control studies. With more than 2900 putative TI peptides available it is likely that the method will be applicable to other types of cancer.