Does Heat Treatment of Human Serum Affect the Measurement of Autoantibodies to Recombinant Tumour-Associated Antigens?

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Aim

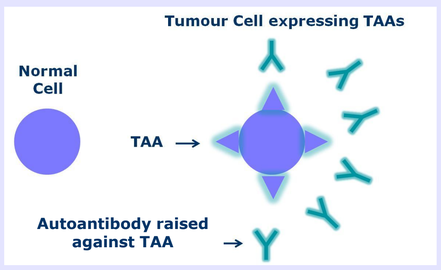
To investigate the effect of serum heat inactivation on the autoantibody response, in order to determine whether or not virally infected serum could be safely screened for autoantibodies using semi-automated assay platform technology.

Heat inactivation at 56°C for 30 minutes is a precautionary step commonly used prior to handling infectious serum.

Background

Mutated, over-expressed, aberrantly expressed or post-translationally modified tumour associated antigens (TAAs) in cancer can often elicit a detectable autoimmune response.

The literature widely documents the presence of circulating autoantibodies raised to autologous tumour-associated antigens in cancer patient serum[1,2].



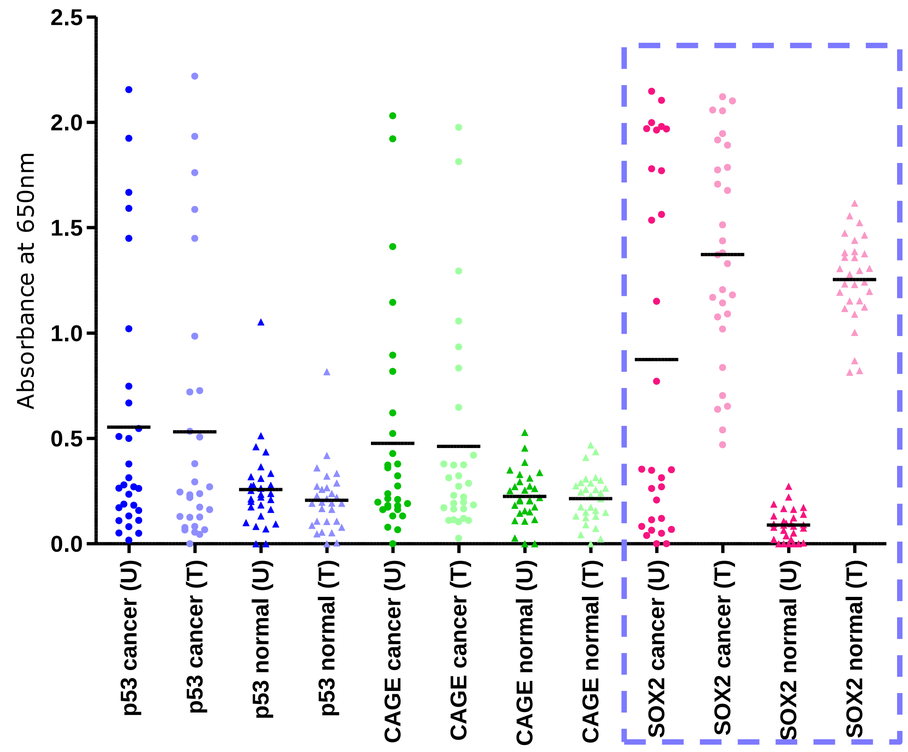
Autoantibodies can provide an *in vivo* amplification of carcinogenesis, in some cases months to years before the tumour becomes otherwise clinically detectable[3]. Autoantibodies have been reported as being of diagnostic potential in lung, breast, colorectal, ovarian, gastric, oesophageal, hepatocellular (HCC) and prostrate carcinomas . Some cancers, such as HCC, are also associated with pre-cancerous viral infections such as Hepatitis B and C Virus. Autoantibody testing is ideally suited to 'at-risk' groups in the population who are pre-disposed to developing cancer.

Methods

Recombinant TAAs p53, CAGE, SOX2 and a control protein (VOL) were produced in *E.coli* and purified according to in house protocols. For the purpose of this pilot study, serum from 29 individuals with Small Cell Lung Cancer (SCLC) (previously identified as having autoantibodies to at least one of these TAAs) and 29 normal human serum samples were subjected to heat inactivation at 56°C for 30 minutes (T) and no-treatment (U). Semi-automated Enzyme Linked Immunosorbent Assay was used to analyse the IgG autoantibody response to a titrated concentration of these antigens. Samples were assessed in duplicate on 2 occasions.

Results

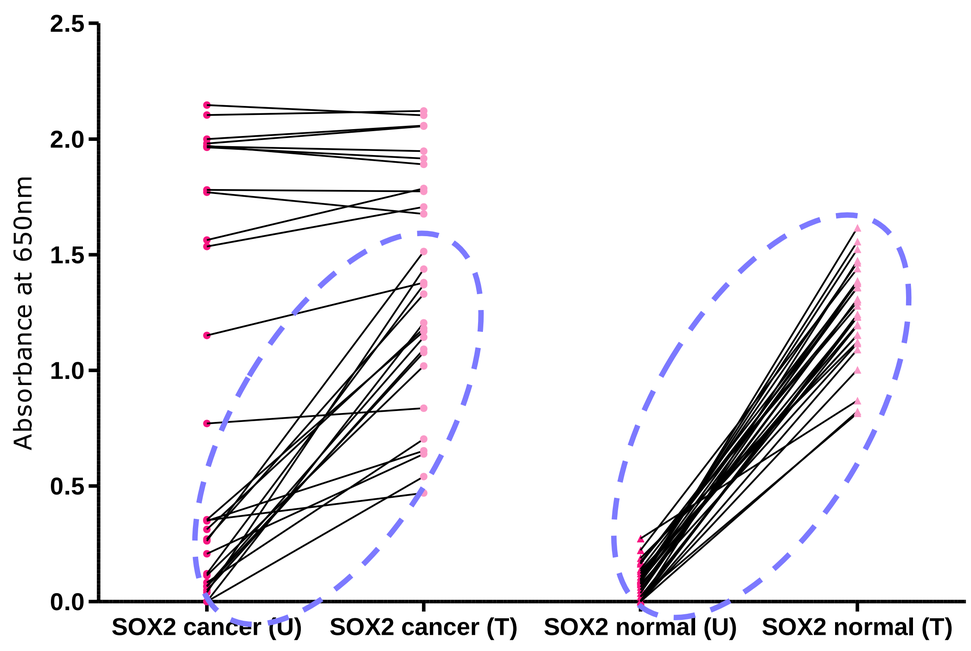
Figure 1 Scatter plots of autoantibody responses to p53, CAGE and SOX2



SCLC and normal samples, untreated (U) and treated (T) at 56°C for 30 minuntes

- No changes in IgG responses to CAGE and p53 were observed after heat treatment.

Figure 2 Autoantibody response to SOX2 at 160nM



SCLC and normal samples, untreated (U) and treated (T) at 56°C for 30 minuntes

- Significantly increased autoantibody responses to SOX2 were observed in both cancer and normal sera following heat treatment ($p<0.001$).
- Increased autoantibody response to SOX2 in heat treated serum was most prominent in normal serum samples and cancer samples with weak autoantibody responses.

Discussion and Conclusion

Initial data suggest that heat inactivation of human serum can considerably affect the autoantibody response to some TAAs.

The elevated autoantibody response observed in heat treated serum is of particular relevance in cases whereby the earlier detection of cancer is reliant upon the detection of autoantibodies in 'at risk' virally infected populations (where heat inactivation of serum is routinely carried out).

Heat treatment of serum should not be performed prior to analysis of autoantibodies to TAAs in serum.

Acknowledgments

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References

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