**Immuno-modulating effects of hyperthermia on PD-L1 and NLRC5 in ovarian cancer**

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**Introduction**

- Ovarian cancer is the leading cause of gynecologic cancer deaths.
- Hyperthermia (HT) as an adjuvant to chemotherapy improves the survival of patients with advanced ovarian cancer through enhancing drug delivery and altering cell metabolism.
- Hyperthermia has also been shown to stimulate the anti-tumor immune response. The main molecular actors behind this effect are heat shock proteins (HSP).
- However, successful anti-tumor immune response is often impeded by immune evasion and suppression mechanisms.

**Methods**

I. 
- WST-8 assay
- Expression pattern of HSP27, 70, 60 and 90

II. 
- Flow separation
- PBMC peripheral blood mononuclear cells
- mRNA/protein extraction
- qPCR and western blot analysis
- PD-L1, NLRC5, IL-10, IL-6, TNF gamma, TGF α, TGF β

III. 
- Analysis of the correlation between PD-L1 and NLRC5 in the TCGA datasets.

**Results**

1. The effect of hyperthermia on cell viability in IGROV1 and SKOV3 cell lines. A considerable increase in viability levels in both cell lines 24h post exposure to 42 °C over 1h, this could be attributed to the development of thermotolerance through HSPs induction. Exposure to 43°C caused a decline in viability in a time-dependent manner.

2. Relative mRNA and protein expression of HSP27 and HSP70 following HT (42°C for 1hr). HSP27 and HSP70 expression increased markedly by 2h post exposure to 42°C and continued to be induced at 24h.

3. Hypothermia attenuates concurrently PD-L1 and NLRC5 gene expression levels in ovarian cancer cell lines in coculture with PBMC. quantitative PCR analysis of the indicated genes mRNA levels in IGROV1 and SKOV3 cells co-cultured with PBMC exposed to 42°C for 1h, followed by incubation at 37°C for 24h. Data were normalized to β-actin and are represented as a fold change compared with coculture at 37°C.

4. Conversely, Hyperthermia induced an increase in PD-L1 and NLRC5 gene expression levels in ovarian cancer cell lines when cultured in the conditioned medium obtained from heat treated cells. Quantitative PCR analysis of the indicated genes mRNA level in IGROV1 and SKOV3 cells cultured under the conditioned media from heat shocked or not shocked IGROV1 and SKOV3 respectively after incubation for 24hrs. Data were normalized to β-actin and are represented as a fold change.

5. Positive correlation between PD-L1 and NLRC5 levels from the TCGA dataset in ovarian cancer. Scatter plot of data points from TCGA ovarian cancerous and normal tissues database, showing that this pair of genes the Pearson coefficient R in ovarian cancerous tissues is 0.54 and is highly significant (P<0.001), but is not significant in normal tissues (R=0.16, p=0.14).

6. Inhibition of HSPB1 (encodes HSP27) and HSPA1A (encodes HSP70) genes with siRNA resulted in a slight decrease in PD-L1 and NLRC5 gene expression in IGROV1 when cocultured with PBMC following heat exposure but this decrease is more pronounced in cells cultured under the conditioned medium obtained from heat shocked transfected with siRNA IGROV1 cell line.

**Discussion**

- Ovarian cancer cell lines coculture with PBMC led to a concomitant decrease in PD-L1 and NLRC5 expression whereas exposure to the conditioned medium by heat shocked ovarian cancer cell lines induced the opposite effect with a significant increase in both PD-L1 and NLRC5 expression.
- PD-L1 and NLRC5 expression is therefore positively regulated by a factor that is secreted from tumor cells upon heat shock and that is however inhibited when PBMC are present.
- Knockdown of HSPB27 with siRNA resulted in a significant decrease of both genes expression.

**Conclusions**

Our findings revealed that hyperthermia modulates the expression of PD-L1 and NLRC5 in a positively correlated manner, and that HSPB27 inhibition could reverse this effect.

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**Disclosure**

The authors declare no conflicts of interest