Exploring the interactions of Interferon-gamma and polyphenols in colorectal cancer cells

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Figure 1: A simplified signal pathway of the induction of PD-L1 by IFN-γ and the subsequent immunoinhibitory response.

Methods

Cell culture - Patient matched Primary adenocarcinoma SW480 and metastatic SW620 human colorectal cancer (CRC) cell lines were cultured in DMEM supplemented with 10% (v/v) fetal calf serum and 100 μg/ml Penicillin-streptomycin and maintained at 37°C with 5% CO₂.

Cell surface staining - PD-L1 was fluorescently stained using anti-human PD-L1 APC antibodies and assayed by flow cytometry (BD FACs Calibur) data was analyzed using FlowJo software.

Cell cycle analysis - Cell cycle analyses were performed on both colorectal cell lines using propidium iodide staining and flow cytometry. Data was analyzed using the Deans-Jets-Fox model and manually set gates.

Cell titre gly assay - Cells were incubated with 20ng/ml of IFN-γ, 0-500 μM of apigenin and 0-500 μM of quercetin for 24hrs. Treatment data was normalized to the 0.1% ethanol vehicle controls which was assigned 100% cell viability.

Statistical analysis - Normality of all data sets was statistically analysed by a Shapiro-Wilk test. Statistical analysis was made using a Kruskal-Wallis with a Conover Inman post hoc test. Results were considered statistically significant when P<0.05.

Results

Interferon-gamma induces PD-L1 in SW480 & SW620 CRC cell lines

IFN-γ induced a significant upregulation of PD-L1 expression on the primary and metastatic colorectal cell lines after 24 hrs compared to unstimulated controls (Figure 2).

Preliminary data of the effect of interferon-gamma primary and metastatic CRC cell cycle progression

The effect of combination treatment of IFN-γ and apigenin on cell cycle progression

Figure 5: Cell cycle distribution was analysed at 24, 48 and 72 hrs after treatment of vehicle control, IFN-γ 20ng/ml, apigenin (Ap) 25 μM or IFN-γ 20ng/ml + apigenin 25 μM (IFN-γ + Ap). Analysis is representative of triplicate wells, each bar represents mean ± standard deviation, symbol * denotes statistical significance (P<0.05).

Conclusions

We found that IFN-γ 20ng/ml is a sufficient dose to stimulate the increased expression of PD-L1 in both the primary and metastatic human colorectal cancer cell lines. This upregulation of PD-L1 in response to IFN-γ stimulation is a strategy for the CRC cells to evade the immune system. The same dose of IFN-γ however was not significant enough to effect the cell cycle or cell viability of the either SW480 or SW620 cell lines.

The polyphenol apigenin displayed a significantly lower, significant dose in the SW480 cell line than quercetin, although had no apparent effect on the metastatic SW620 cell line. However, quercetin did lower ATP production in the SW620 cell line at 250 μM. These data suggest the two polyphenols have potential anti-cancer properties, however, apigenin has shown to be more potent in the primary SW480 cell line. Polyphenols could be considered as effective adjuvants to immunotherapies.

Future research

With funding from the British society of immunology we are looking to continue researching the combination effect of IFN-γ and apigenin on PD-L1 expression, apoptosis and further cell cycle analysis.

Apigenin has been shown to be an inhibitor of STAT1 and STAT3 (Hui Hui Cai, et al 2016), so we hypothesise that apigenin may be able to reduce the level of PD-L1 induced by IFN-γ.

References


Interferon-gamma and polyphenols in colorectal cancer cells

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Figure 2: (A) Representation of gated SW480 cells and PD-L1 expression histograms. (B) Both SW480 and SW620 cells were incubated with 20ng/ml of IFN-γ for 24, 48 and 72 hours, and the median fluorescent intensity (MFI) was compared against the MFI of unstimulated control (Control). Treatments were run in triplicate in two independent experiments; data is expressed as mean ± standard deviation. Statistical significance was set at P<0.05 and is denoted **.

Figure 3: Cell cycle distribution was analysed at 24, 48 and 72 hrs when unstimulated or treated with IFN-γ 20ng/ml. Analysis is representative of triplicate wells, each bar represents mean ± standard deviation, symbol * denotes statistical significance (P<0.05).

Figure 4: The effect of apigenin and quercetin on the ATP levels as a measure of cell viability in SW480 and SW620 cells after 24 hours; evaluated by cell titre gly assay. Data was normalised to the vehicle control (VC) mean which was assigned 100% cell viability. Data expressed as median and ranges. Statistical significance was determined by comparison with the vehicle control, and statistical significance was set at P<0.05 which was determined as the lowest significant dose.

Apigenin displayed a lowest significant dose of 25 μM in the primary SW480 CRC cells (Figure 4(A)) but no effect was seen in the metastatic SW620 CRC cells when treated with a dose up to 500 μM (Figure 4B).

Quercetin displayed a lowest significant dose of 250 μM for both CRC cell lines (Figure 4C & 4B).

Apigenin was chosen for further experimentation as it had the most physiologically relevant lowest significant dose.