Sharply discordant biological properties of synthetic noncoding dsRNA of different size: translational opportunities in cancer

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Background

- Noncoding dsRNAs stimulate immunity and are capable to induce cell death in certain types of cells by engaging various signal transduction pathways through TLRs, MDA5 and RIG-I.
- Depending on the chemical structure and molecular weight, synthetic RNAs could differentially trigger these and additional pathways yet to be characterized, providing an opportunity to discover, optimize and translate novel immune interventions for hepatocellular carcinoma and other unmet medical needs.
- Earlier studies showed that unfractionated polyA:polyU spanning low and high molecular weight molecules (5bps to >100bps of dsRNA) effectively licensed antigen presenting cells to cross-prime Tc1 responses and facilitated efficacious anti-tumor immunity [1, 2]

Aims

- To evaluate polyA:polyU of different molecular sizes, obtained by size-fractionation, relative to the capability to induce cytokine production and cell death.
- To advance and test synthetic polyA:polyU generated to precise specifications in terms of molecular size, in regards to activity on human hepatocellular carcinoma cells.

Methods

- Synthetic polyA:polyU of heterogenic size from Sigma was endotoxin-purified and size fractionated by centrifugation through membranes of specified MW cutoff (Amicon).
- As an alternate manufacturing approach, the polyA:polyU was generated to a pre-specified size of 5bps (low molecular weight – LMW) or 70bps (high molecular weight – HMW) by Sigma and Midland Certified respectively. 2O'-methylated versions of polyA:polyU were also synthesized and tested.
- The following cell types and lines were used: human monocyte cell line (THP-1), human liver cancer cell lines (Huh7, PLC/PRF/5, HepG2), primary human liver cancer cells, and other cells as controls: primary human fibroblasts, mouse liver cancer cell line (BNL 1.ME A.7R.1).
- Cytokine production (TNFalpha and IL-12p70), was measured by ELISA (R&D systems) (Fig.1).
- The following methods to measure the effect on cell proliferation, death and apoptosis were used: EB, PI and YoPro staining (Fig. 1), MTT assay (Fig. 2), Annexin V and PI staining analyzed by flow cytometry (Fig. 3).

Fig 1. Effect of fractionated pA:U on THP-1 cells

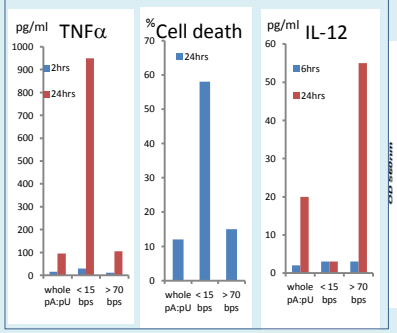
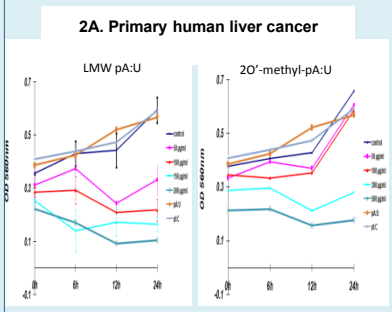
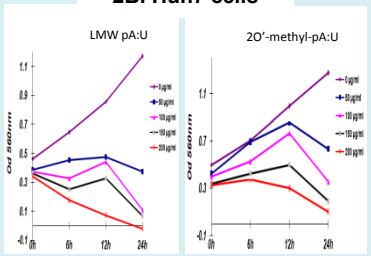
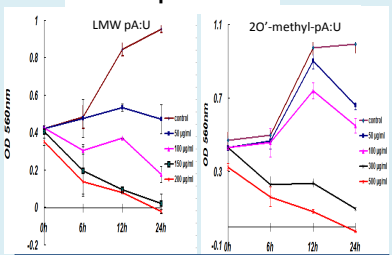


Fig 2. Anti-proliferative effect of 5bp pA:U (MCT485)







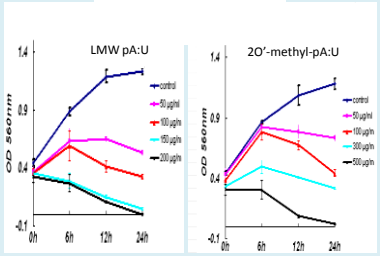
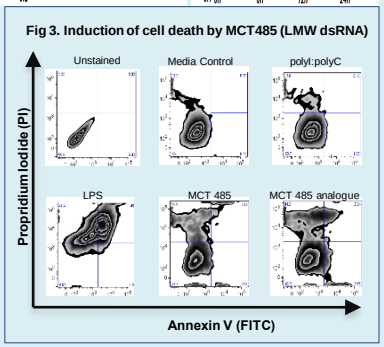


Fig 3. Induction of cell death by MCT485 (LMW dsRNA)



Results

- Size fractionation of heterogenous polyA:polyU yielded low molecular weight dsRNA (<15bps) with substantial TNFalpha and cell death inducing properties in human monocytic THP-1 cells.
- In sharp contrast, size fractionated polyA:polyU of high molecular weight (>70bps) induced high levels of IL-12p70 in human monocytic THP-1 cells, with minimal cell death or apoptosis.
- Synthetic low molecular weight polyA:polyU of 5bps induced substantial cell growth inhibition and death in three distinct human hepatocellular carcinoma cell lines, and primary liver cancer cells in a dose-effect fashion.
- 2O'-methylated polyA:polyU of 5bps showed an attenuated cytotstatic / cytotoxic profile.

Conclusions

- Starkly different biological effects of polyA:polyU are mediated by species with distinct size, reminiscent of engaging distinct receptors and pathways: low molecular weight dsRNA of 5bps is intensely cytotoxic (inducing necrosis or pyroptosis) and high molecular weight dsRNA is immune modulating through IL-12 induction.
- Thus, by creating synthetic dsRNA of pre-specified larger size, one could enhance its IL-12 dependent immune modulating properties and further diminish or eliminate any potential liabilities, building on previous clinical experience [3, 4] with safer and more potent compounds.
- Conversely, this paves the way to generating novel and potent cytotoxic agents – synthetic dsRNA of reduced size - with different mechanism of action, requiring targeted delivery to tumors.
- A comprehensive preclinical evaluation of synthetic short dsRNA (MCT 485) and longer sized dsRNA (MCT 465) of defined size and chemistry, for potential translation to liver cancer and other diseases, has been initiated.

References

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