DNA-based Polyvalent Antibody Mimic for Immunoengineering of Natural Killer Cells

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Statement of Purpose: DNA molecules have been utilized as building units to synthesize various nanostructures due to their nature of programmable and predictable hybridization. In this study, an aptamer-based polyvalent antibody mimic (PAM) was synthesized on the surface of natural killer (NK) cells. The PAM is formed on cell surface through hybridization chain reaction. The results show that PAM-functionalized NK cells acquire higher efficiency of binding cancer cells and therefore kill cancer cells with improved efficiency compared to monovalent aptamers. Since the DNA sequences used in this study can be substituted with other functional ligands during the PAM synthesis, this work demonstrates a novel DNA nanostructure-based platform with versability in promoting immune and cancer cell interactions for cancer immunotherapy.

Methods: The oligonucleotides for DNA-based polyvalent aptamer engineered antibody mimic (PAM) nanostructure synthesis were purchased from Integrated DNA Technologies (Coralville, IA). The synthesis of the PAM will be performed using similar procedures as previously reported. In brief, the cholesterol conjugated DNA initiator (DI) was incorporated to the cell membrane through lipid insertion. DNA monomer 1 (DM1) and DNA monomer 2 with branched sequence (DM2) were added together to form DNA template via hybridization chain reaction (HCR). Then, DNA aptamer sequence was added to form the PAM nanostructure. The successful formation of PAM nanostructure by hybridization chain reaction was characterized using gel electrophoresis. Confocal microscopy and flow cytometry were used to examine the successful PAM formation on the cell surface. In this study, DNA-based monovalent aptamer engineered antibody mimic (MAM) was used as conventional method to be compared with PAM for enhanced cell-cell interaction. NK cells and K562 cells were used in this study for the representative cell-cell interaction analysis. Fluorescence microscopy and flow cytometry were used for the examination of improved binding efficiency between either PAM or MAM engineered NK cells and K562 cells. Propidium iodide (PI) and flow cytometry were utilized to investigate the K562 killing efficiency of NK cells.

Results: Gel electrophoresis results showed that the DNA-based nanostructures were synthesized from single stranded oligonucleotide sequences. Confocal laser scanning microscopy images demonstrated the formation of PAM mainly on the cell surface. In addition, flow cytometric analysis illustrated the increase of fluorescence intensities, demonstrating the repeats of DNA units during the HCR process. The fluorescence intensities of PAM modified NK cells were eight times amplified for the DM1-Cy5 sequence during the HCR and seven times amplified for the aptamer-FAM sequence signal compared to MAM modified NK cells. Fluorescence microscopy images

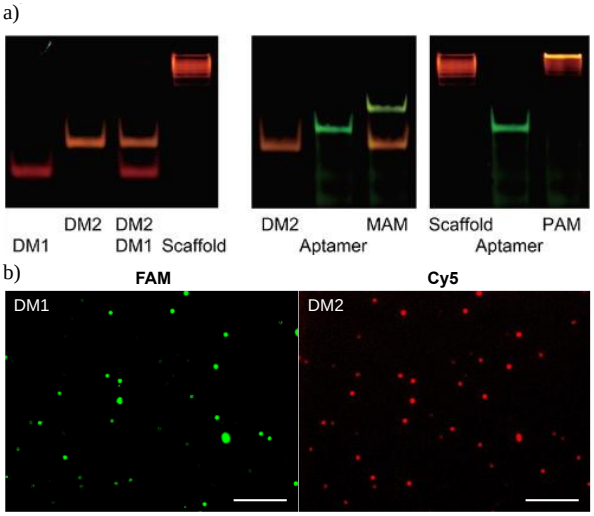

Figure 1: *a) Electrophoresis gel images showing successful formation of MAM and PAM . b) Fluorescence microscopy images of PAM modified microparticles using DM1-FAM and DM2-Cy5. Scale bars represent 50 μm.*

showed that PAM modified NK cells could bind to K562 cells more efficiently than native NK cells, forming cell-cell clusters in ratio of 1:1, 1:2, and 1:3. In addition, flow cytometric analysis demonstrated the enhanced cell-cell interaction of PAM modified NK cells with K562 cells. Native NK cells, MAM modified NK cells, and PAM modified NK cells were bound to 26%, 40%, and 74% of K562 cells, respectively. The calculated enhancement efficiency of the MAM modification and PAM modification were 54% and 185%, respectively. The efficiency of native NK cells, MAM modified NK cells, and PAM modified NK cells for killing K562 cells were 7.9%, 16.3%, and 29.5%, respectively.

Conclusion: Aptamer-based polyvalent antibody mimic was successfully synthesized on the NK cell surface through sequence hybridization using precisely programmed oligonucleotide sequences. The polyvalent aptamers on NK cell surface led to enhanced interactions of immune and cancer cells. Resultantly, the cancer cell killing efficiency was significantly enhanced. Thus, this study successfully demonstrated that the synthesis of PAM on NK cells is a promising strategy for improving the efficiency of cancer immunotherapy.

References:

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Acknowledgements: This work was supported in part by the U.S. National Science Foundation (CBET 1802953; DBI 1911764).