4623

Human Anti-Nucleolin Antibodies with Broad Spectrum Anticancer Activity

Daniel J. Fernandes[1,2], Yoko Otake[1], Natalie A. Sutkowski[2] and Robert L. Capizzi[1]
[1]CharlestonPharma, LLC, Charleston, SC. [2]Medical University of South Carolina, Charleston, SC.

BACKGROUND

Nucleolin

- **Nucleolin is a multifunctional phosphoprotein that is highly overexpressed in the plasma membrane and cytoplasma of most tumor cells, but is usually undetectable on the surface of the normal cells.**
- **Overexpression of nucleolin on the surface of many types of tumor cells accounts for the broad-spectrum anti-tumor activity of the anti-nucleolin HuMAbs.**
- **Anti-nucleolin HuMAbs gain intracellular access possibly by nucleolin-mediated endocytosis.**

Human Antibody Technology

Figure 1. Supplying agents that mimic antigen and T cell help overrides the EBV growth program, and elicits germinal center functions, like isotype class switching and IgG secretion. This creates an "*in vitro* germinal center" for immortalized human B cells, which was adapted for Hu Mab production.

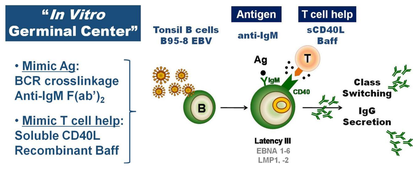


Figure 2. EVB Infection and IgG Induction. (A) Tonsil B cells were infected with GFP-labeled EBV (EBfaV-GFP) either by traditional supernatant infection or spinfection with 10x concentrated virus. **(B)** Tonsil B cells (n=8) were spinfected with 10x concentrated B95-8 EBV in presence or absence of anti-IgM, sCD40L, and Baff. Cell sups were collected on day 5 and 15, and ELISA was performed for IgM and IgG. IgG was induced as early as 5 days post-infection.

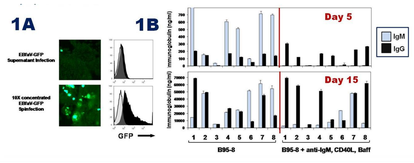


Figure 3. A Novel Platform for Isolating Fully Human Antibodies *In Vitro*. Tonsil B cells are efficiently immortalized with EBV, then induced to secrete IgG. Immortalized B cell libraries are plated into multiple wells, then cell supernatants containing IgG are screened for binding to an antigen of interest, here the auto-antigen nucleolin. Reactive cells are isolated by limited dilution cloning, and expanded for IgG purification.

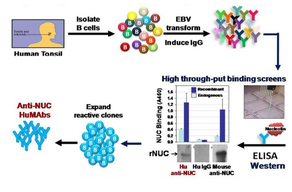


Figure 4. Differential Cell Surface and Cytoplasmic Expression of Nucleolin in Mammary Carcinoma and Normal Mammary Epithelial Cell Lines. The intracellular localization of nucleolin was determined by indirect immunofluorescence using mAb against human nucleolin and FITC-conjugated secondary Ab. Nuclei were counterstained with propidium iodide. Cell morphology is shown as DIC images. **(A)** MCF-7 breast cancer and MCF-10A normal mammary epithelial cells. (Soundarajan *et al*, Cancer Res. 2008, 68:2358). **(B)** MDA-MB-231 breast cancer cells. Results are representatives of three experiments.

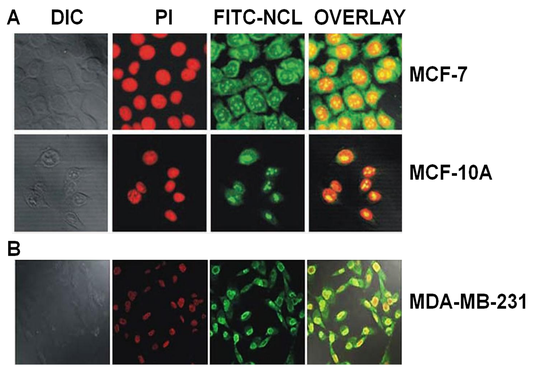
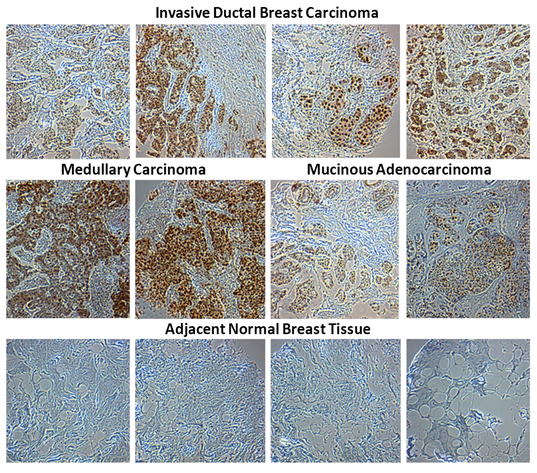


Figure 5. Nucleolin Overexpression in Human Breast Carcinomas. A tissue array of breast tumor and adjacent normal tissue (Biomax BR1006) was stained with anti-NCL (MS-3 antibody, 1:100) with Vectastain mouse kit, DAB based peroxidase substrate and hematoxylin counter-stain. Each panel is a specimen from a different patient. Representative specimens are depicted. 100x magnification.



RESULTS

Broad-Spectrum Anti-Cancer Activity

Figure 6. Nucleolin Specific Killing of MCF-7 Breast Cancer Cells Occurs Without Toxicity to MCF-10A Normal Mammary Cells. (A) Surface and cytoplasmic over-expression of nucleolin (FITC-labeled) in MCF-7 *vs*. MCF-10A cells. Nuclei were counter-stained with propidium iodidie (PI). **(B)** Nucleolin specific HuMAbs CP101 and CP201 were tested for binding to recombinant nucleolin (10 or 100 ng) by ELISA assay. HuMAbs were added at concentrations ranging from 0-400 ng/ml and binding was detected with HRP labeled anti-human IgG and DMB substrate. **(C)** MCF-7 and MCF-10A cells were treated with HuMAbs CP101 or CP201. Viability was assayed at 96h by trypan blue counting. **(D)** MCF-7 cells were incubated with anti-NCL HuMAbs (2μg/ml) in the presence of heat inactivated serum (- complement) or human AB serum (+ complement). Viability was determined by MTS assay at 48 - 96h.

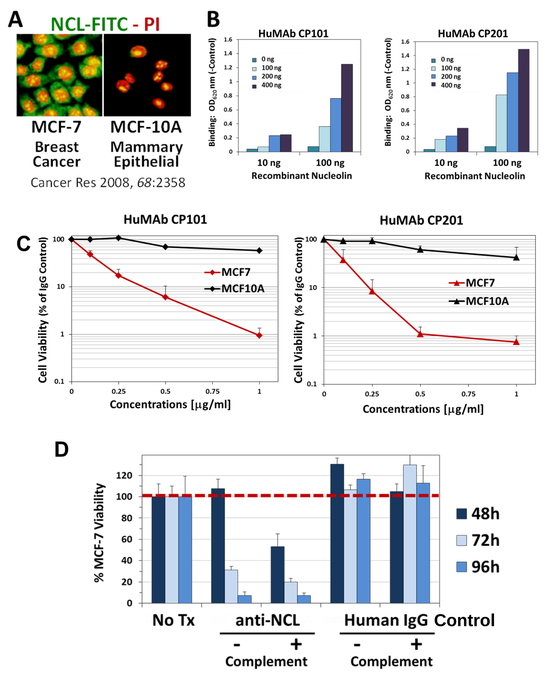


Figure 7. Effects of CP101 and CP201 HuMAbs on the Growth of Prostate and Pancreatic Cancer Cells. Growth inhibition was determined as described in Figs 6 and 8. Results are the means of 4 experiments ± SD.

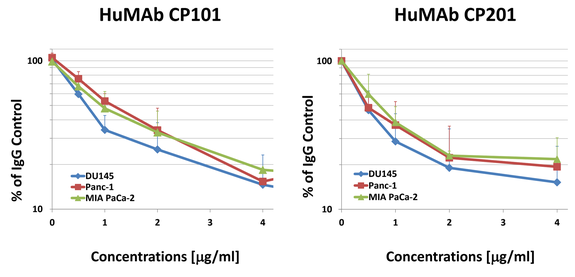
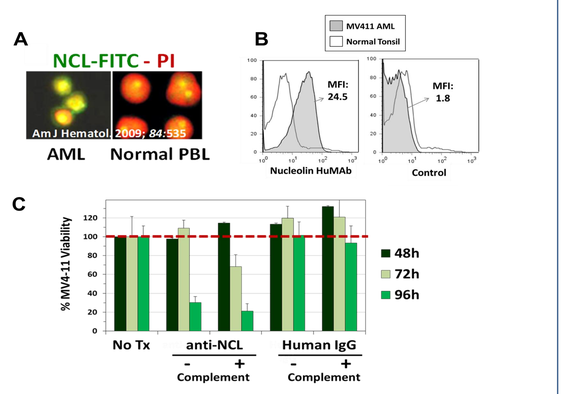


Figure 8. HuMAb Binding to Cell Surface Nucleolin and Killing of MV4-11 Leukemia Cells. (A) Intracellular localization of nuclelin in AML and normal PBL was shown by indirect immunofluorescence, using mouse anti-NCL MS3 MAb and FITC-anti-mouse IgG. Nuclei were counterstained with propidium iodide (PI). **(B)** MV4-11 and normal tonsil cells were stained for flow cytometry with anti-NCL HuMAb (5μg/ml), followed by APC-labeled anti-human IgG. Median fluorescence intensity (MFI) is indicated. **(C)** MCF-7 cells and MV4-11 cells were incubated with anti-NCL HuMAbs (2μg/ml) in the presence of heat inactivated serum (- complement) or human AB (+ complement). Viability was determined by MTS assay at 48 – 96h.



CONCLUSIONS

- **The fully human anti-nucleolin HuMAbs CP101 and CP201 bind tightly to human recombinant nucleolin and to nucleolin on the surface of tumor cells.**
- **CP101 and CP201 can kill tumor cells independently of CDCC and HDCC although complement increases the rate of cell killing.**
- **Direct tumor cell killing by CP101 and CP201 is consistent with intracellular uptake of the HuMAbs.**
- **The broad spectrum activity and high tumor cell selectivity of CP101 and CP201 against common solid tumors and acute leukemias suggest that anti-nucleolin HuMAbs have a large market potential.**

ACKNOWLEDGEMENTS

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