Identification of depressed HLA class I expression and CD44^bright/CD24^dim as the phenotype of floating but not adherent subpopulation harboring most tumor-initiating cells in the UP-LN1 carcinoma cell line

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Abstract

UP-LN1 is a poorly differentiated carcinoma cell line established from a lymph node (LN) metastatic lesion of the neck of a male patient with unknown primary. The C7K/C2K0/CEA/SCCA phenotype detected in the original metastatic lesion and cultured cells led us to believe this cell line to be originated from a primary cancer of the gastrointestinal tract. UP-LN1 exhibited unique in vitro growth characteristics with naturally occurring floating (F) and adherent (A) cells. We hypothesized that the tumor-initiating cells (TICs) with cancer stem cell (CSC) properties may have differentially distributed in F and A cells of the UP-LN1 cell line contributing to cancer metastasis. We first made comparisons of TIC properties between F and A cells in terms of tumorigenicity in NOD/SCID mice. F and A cells were further examined for the expression of selected tumor markers and genes associated with TICs by DNA-microarray, RT-PCR and cytofluorometric analyses. Comparative sensitivities of the two cell types to cytotoxicity of non-MHC-restricted effector cells were also determined using [3H]cytotoxicity release assay.

1. To characterize the distribution and phenotype of tumor-initiating cells in A and F subpopulation within the UP-LN1 carcinoma cell line.

2. To determine if the A (adherent) and F (floating) phenotypes identified in vitro can be extrapolated in the patient's lymph node metastatic lesion regarding the loose and compact tumor cells.

3. To illustrate the interaction between tumor-initiating cells and immune effector cells in the lymph node metastasis microenvironment.

Fig 1. Representative morphology of five cultures of parental UP-LN1 cell line (A) and isolated A (B) and F (C) subpopulations. *A cells = (CD56dim/CD16+cytotoxic NK cells).

Fig 2. Differential HLA class I and CD44/CD24 expression profiles of A and F cells in culture and original tumor lesion biopsy. The expression of HLA class I (A) with or without IFN-γ treatment and the CD44/CD24 expression profile (C) of cultured P, A, and F cells was assayed by flow cytometric analysis. In contrast to moderate HLA class I expression and the CD44^bright/CD24^dim phenotype of A cells, F cells showed a distinct CD44^dim/CD24^bright phenotype and down-regulated HLA class I expression. Also, the CD44^dim/CD24^bright phenotype could be found in the region with loose tumor cell structure.

Fig 3. Xenotransplantation experiments of P, A, and F cells in NOD/SCID mice. P, A, and F cells were injected into NOD/SCID mice with different number from 10⁵ to 10⁶ (with 10-fold dilution) per mouse. Each group of 5 mice was used for xenotransplantation. *Number of animal with tumor / number of animal injected.

Fig 4. The HLA class I and CD44/CD24 expression profiles of P, A, and F cells recovered from xenograft tumors in NOD/SCID mice. Xenograft tumors derived from P, A, and F cells were dissociated and cultured for demonstration of the ability of each cell type to differentiate and generate the parental phenotypes. Cultured cells from respective tumors resulting from P, A, and F cells were assayed for surface expression of HLA class I, CD44, and CD24 by triple-color flow cytometric analysis. Results show each of A and F cells were able to regenerate both cell types to behave like parental cells in vivo.

Fig 5. Relative susceptibility of P, A, and F cells to purified CD56^bright/CD16^cytotoxic NK cells. CD56^bright/CD16^cytotoxic NK cells with ≥ 95% purity (A) were isolated from PBMCs of healthy donors by magnetic-beads and activated with IL-2 before they were added to the 3E2 release cytotoxicity assay. The susceptibility of P, A, and F cells to activated CD56^bright/CD16^cytotoxic NK cells were revealed by % specific lysis (B, left). The NK-92 and K562 cells were served as reference targets of NK cells in each assay (B, right).

Table 1. Tumor initiating cell frequencies of A and F subpopulations of the UP-LN1 cell line as revealed by xenotransplantation in NOD/SCID mice

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<th>Tumor incidence</th>
<th>TIC frequency *</th>
<th>95% confidence interval</th>
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<tr>
<td>P</td>
<td>5/5</td>
<td>(1/4542 – 1/4560)</td>
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<tr>
<td>A</td>
<td>5/5</td>
<td>(1/10^4 – 1/10^5)</td>
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Results

1. In contrast to the moderate HLA class I expression and the CD44^bright/CD24^dim phenotype of A cells, F cells showed a distinct CD44^dim/CD24^bright phenotype and down-regulated HLA class I expression. Also, the CD44^dim/CD24^bright phenotype could be found in the region with loose tumor cell structure.

2. To determine if the A (adherent) and F (floating) phenotypes identified in vitro can be extrapolated in the patient's lymph node metastatic lesion regarding the loose and compact tumor cells.

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Summary

1. In contrast to the moderate HLA class I expression and the CD44^bright/CD24^dim expression profile of A cells, F cells exhibited further down-regulated HLA class I expression and the CD44^dim/CD24^bright phenotype, which have been well characterized as breast as well as pancreatic cancer stem cells.

2. The floating and attached cells detected in cultured UP-LN1 cells seem to be consistent with the loosely attached and compact cells formed in the patient's original lymph node lesion, based on the immunofluorescence/confocal microscopy imagination.

3. As revealed by xenotransplantation with serially diluted A and F cells in NOD/SCID mice, TICs showed to be 11.5 times more enriched in F subpopulation than in A subpopulation.

4. Only TICs-F cells but not A cells displayed extreme resistance to IL-2-activated CD56^bright/CD16^cytotoxic NK cells and plasticity for induction of metastatic cancer stem cells by IFN-γ-mediated enhancement on metastatic potential modulation of the CD44^dim/CD24^bright phenotype.

5. The UP-LN1 cell line provides an ideal cell model for further studies in (i) transformation and hierarchy of cancer stem cell, and (ii) interactions between immune effector cells and cancer stem cells in a metastasis microenvironment, such as lymph nodes.

Conclusion

The observation of IFN-γ-induced CXCR4-mediated/migratory CSC subset from F cells in this study implies that immunotherapy with non-MHC-restricted effectors such as LAK and activated NK cells will not be advised for cancer patients in whom the expression of CXCR4 is inducible by IFN-γ. Whether MHC-restricted T cell-based immunotherapy can be used to eradicate CD44^bright/CD24^dim tumor cells is yet to be determined.

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