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Aging Increases Susceptibility to Ovarian Cancer Metastasis in Murine Allograft Models and Alters Immune Composition of Peritoneal Adipose Tissue[1]

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Abstract

Ovarian cancer, the most deadly gynecological malignancy in U.S. women, metastasizes uniquely, spreading through the peritoneal cavity and often generating widespread metastatic sites before diagnosis. The vast majority of ovarian cancer cases occur in women over 40 and the median age at diagnosis is 63. Additionally, elderly women receive poorer prognoses when diagnosed with ovarian cancer. Despite age being a significant risk factor for the development of this cancer, there are little published data which address the impact of aging on ovarian cancer metastasis. Here we report that the aged host is more susceptible to metastatic success using two murine syngeneic allograft models of ovarian cancer metastasis. This age-related increase in metastatic tumor burden corresponds with an increase in tumor infiltrating lymphocytes (TILs) in tumor-bearing mice and alteration of B cell-related pathways in gonadal adipose tissue. Based on this work, further studies elucidating the status of B cell TILs in mouse models of metastasis and human tumors in the context of aging are warranted.

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Introduction

Ovarian cancer (OvCa) is the leading cause of death due to gynecological malignancy in women in the United States. Often diagnosed with metastases, patients with ovarian cancer receive poor prognoses [1]. The vast majority of OvCas are epithelial in origin with predominantly serous (70%–85%) and endometrioid (10%) histotypes [2,3]. OvCa metastasizes uniquely when tumor cells or multicellular aggregates detach from the primary tumor and disseminate throughout the peritoneal cavity, forming metastatic sites on the peritoneum [1]. Additionally, hematogenous metastasis with metastatic homing to the ovary has been seen in model systems [4,5].

Aging is a significant risk factor and prognostic factor for women with OvCa, wherein the median age at diagnosis is 63 [6]. Furthermore, elderly patients have poorer prognoses, with unfavorable progression-free survival (PFS) and overall survival associated with increased age [7–11]. While treatment disparities in the elderly

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may contribute to this difference, the aged host may also be more susceptible to disease progression. There is a paucity of studies addressing the impact of aging on OvCa metastasis.

The fact that OvCa is so often diagnosed at metastatic stages of the disease confounds the ability of researchers to draw conclusions about the relationship between metastasis and age from patient data. Thus, to evaluate this relationship, research models of metastasis are required. *In vitro* models using human omental mesothelial cells (HOMCs) have demonstrated that HOMCs from elderly patients are in a pro-inflammatory state [12]. Another study showed an increase in adhesion of OvCa cells to HOMCs in a state of induced senescence *in vitro* [13]. While a single ovarian cancer metastasis study has utilized middle-aged mice [14], studies designed to evaluate the impact of age on metastasis *in vivo* have not been reported.

The purpose of this study was to test the hypothesis that age impacts the metastatic outcome of OvCa. Here we utilize distinct syngeneic murine models of OvCa metastasis to demonstrate that age increases the susceptibility of the host to metastasis. Our data suggest that the immune composition of aged peritoneal adipose tissue, specifically B cells, may contribute to this age-related disparity in metastasis.

Materials and Methods

Murine Aging Models

Two age groups of C57Bl/6 female mice (Jackson Laboratories) were used for this study. Retired breeders were purchased around 8 months of age and allowed to age until they reached 20 months, at which point they are devoid of ovarian follicles [15]. Young mice were bred once beginning at 8 weeks and were used between 4–6 months of age. Mouse age time points were chosen based on research on mouse-human lifespan equivalencies [16]. In this murine model of aging, mice 3–6 months (young) and 20–23 months (aged) correspond to humans 20–30 and 60–67 years of age, respectively (Figure 1A). The same methods were used for experiments with FVB/NJ mice (Jackson Laboratories), which have a comparable lifespan to C57Bl/6 mice [17]. All animal procedures were carried out according to the regulations of the Institutional Animal Care and Use Committee at The University of Notre Dame.

Murine Allograft Models

Two allograft models were utilized in this study. Parental ID8 cells, a C57Bl/6 syngeneic mouse ovarian surface epithelial (MOSE) cell

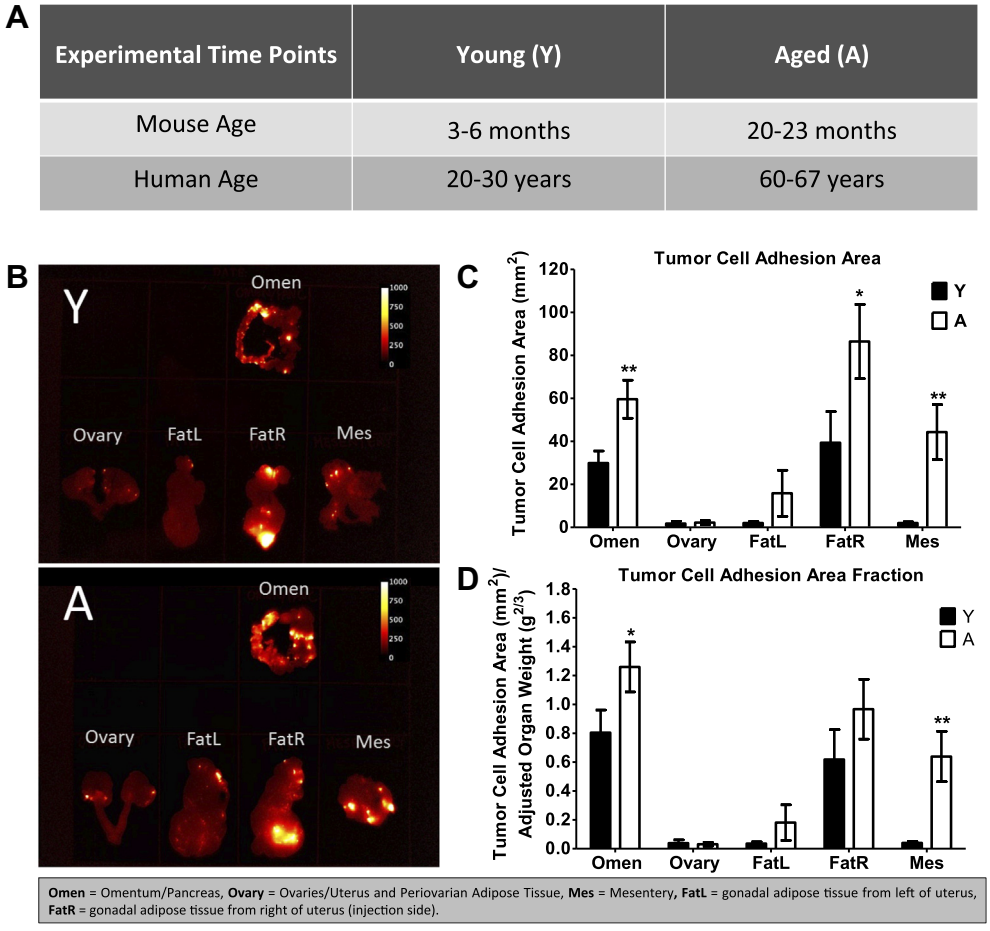

Figure 1. Short-term *in vivo* assay of ID8 cell adhesion to peritoneal adipose tissues. A. Mouse ages and equivalent ages in human years. Mouse ages were chosen based on work by the Harrison Laboratory at Jackson Laboratories [16]. B. Representative tumor cell adhesion images. Young (Y) and Aged (A) mice were injected IP with $4.3x10^6$ ID8 cells tagged with RFP and sacrificed the following day. Major peritoneal adipose depots were dissected and imaged *ex vivo* using the Bruker *In Vivo* Xtreme imaging system. C, D. Quantification of organ-specific tumor cell adhesion. Tumor cell adhesion area (C) was quantified using ImageJ as described in Materials and Methods. For Omen $P = .01$; FatR $P = .05$; Mes $P = .004$. Tumor cell adhesion area fraction (D) was quantified by adjusting for the weight of the organs. N = 10. For Omen $P = .07$; Mes $P = .003$. Double asterisk indicates $P < .05$, single asterisk indicates $P < .1$.