Abstract # 2288 Lipoprotein(a) and vitamin C affect the development of breast cancer tumors in Lp(a)+; Gulo-/- mice

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Objective

Lipoprotein(a), composed of LDL and an adhesive protein apo(a), is produced in humans and primates, the species which lost an ability to synthesize vitamin C endogenously. We have shown earlier that Lp(a), due to its strong ECM binding properties, may be considered a biological 'stability' molecule for the structurally weakened connective tissue in the vascular wall. The development and progression of cancer is characterized by loss of ECM integrity which facilitates tumor growth and metastasis. We developed a unique mouse model lacking endogenous vitamin C production (Gulo-/-) and synthesizing human Lp(a) (lp(a)+), which has been used in this study to investigate the role of Lp(a) and other lipoproteins in cancer.

Methodology

1. The female Gulo-/-:Lp(a)+ and control wild type Balb/c mice were orthotopically inoculated with 4T1 breast cancer cells (500,000)
2. The transgenic and control mice were divided into 4 different dietary groups in respect to dietary vitamin C intake:
   - A) Low ascorbate intake for 6 weeks (GALC1)
   - B) Low ascorbate intake for 3 weeks followed by high ascorbate for 3 weeks (GALC2)
   - C) High ascorbate intake for 6 weeks (GAHC1)
   - D) High ascorbate intake for 3 weeks followed by low ascorbate for 3 weeks (GAHC2)
3. Control groups of Lp(a)+;Gulo(-/-) mice without tumor inoculation were put on the same Vitamin C regimens.
4. Wild type controls included mice without and with 4T1 inoculation were kept on regular mouse chow for 6 weeks.

Vitamin C regimens defined;
Low ascorbate: 30 mg/L vitamin C water and regular diet
High ascorbate: 150 mg/L vitamin C water and 500 ppm diet

Results

1. After 6 weeks, 100% of wild type mice developed tumors, while 50% of Lp(a)+;Gulo(-/-) mice kept on high ascorbate diet for 6 weeks did not develop primary tumors and, in some mice, residual tumor cells or inflammatory infiltrates were detected in the lungs by histology.

Figure 1 - Primary tumor incidence in mouse groups injected with 4T1 cells

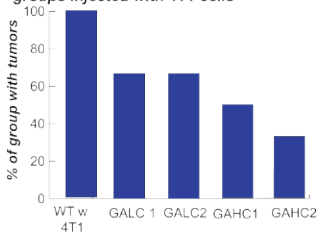


Figure 2 - Serum ascorbate in groups

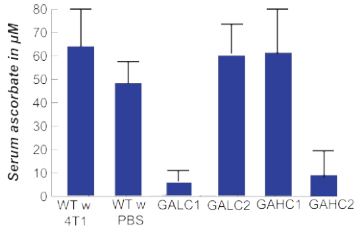


Figure 3 - Total surface lung nodules in groups (n-6)

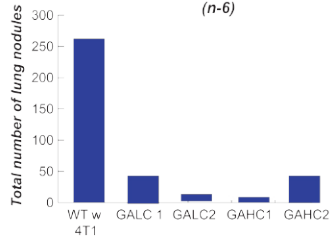
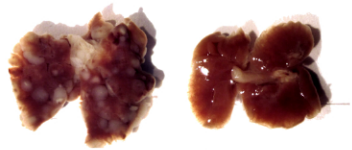


Figure 4 - Representative lungs showing tumor metastasis in groups



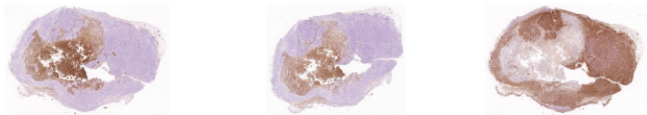
Wildtype mouse (#2)

Gulo-/-;Lp(a)+ high vitamin C (#975)

2. Primary tumors from wild type mice were on average over 2-fold larger (1.80+/-0.62g) than tumors from Lp(a)+;Gulo(-/-) mice on continual low Vitamin C (0.77+/-0.98g) or continual high Vitamin C (0.63+/-1.09g. Primary tumors from Lp(a)+;Gulo(-/-) mice immunostained positively for Lp(a) and their size was inversely proportional to Lp(a) cholesterol serum levels.Lp(a) could not be detected in tumors from wild type mice and the presence of tumors was associated with higher LDL serum levels.

Figure 5 Tumor immunostains of Apo(a), hApo(B) and mApo(B) in Lp(a) mouse tumor compared to WT mouse cholesterol stains

GAHC1 apo(a) GAHC1 hApo(B) GAHC1 mApo(B) (mouse #975)



Wild type apo(a) Wild type hApo(B) Wild type mApo(B) (mouse #2)

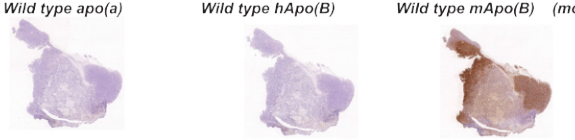
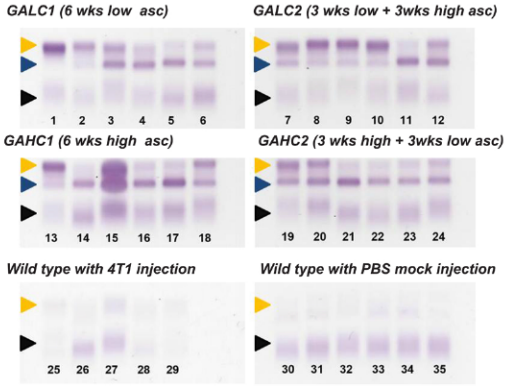


Figure 6 Lp(a)-cholesterol profile panel for mouse groups



Corresponding 4T1 tumor mass in female Gulo-/-;Lp(a)+ mice and WT mice:

6 wks low asc: 1) 2.6g, 2) 0.45g, 3) 1.05g, 4) 0.528g, 5) no primary, 6) no primary

3 wks low + 3 wks full asc: 7) 0.52g, 8) 1.95g, 9) 0.956g, 10) 0.14g, 11) no primary, 12) no primary

6 wks full asc: 13) 0.85g, 14) 2.76g, 15) 0.17g, 16) no primary, 17) no primary, 18) no primary

3 wks full + 3 wks low asc: 19) 3.0g, 20) 0.06g, 21) no primary, 22) no primary, 23) no primary, 24) no primary

Wild type w 4T1 25) 2.0g, 26) 0.95g, 27) 1.8g, 28) 2.1g, 29) 2.7g

PBS mock injected female wildtype mice: 30-35) none

Summary and Conclusion

Primary breast tumor incidence and size, as well as secondary lung metastatic nodules were drastically reduced in Lp(a)+;Gulo -/- mice on ascorbate supplementation compared to Wild type mice. Furthermore, apo(a) and human ApoB were found abundantly in tumors from Lp(a)+;Gulo -/- mice, but were absent from tumors in WT mice. The results implicate that Lp(a) and ascorbate play roles in controlling tumor growth and metastasis.