

第6回アジア造影超音波会議

The 6th Asian Conference on Ultrasound Contrast Imaging (ACUCI 2014)

President

Fuminori Moriyasu

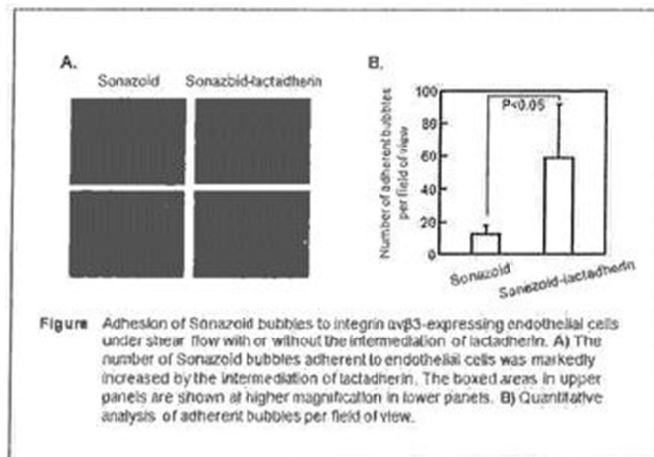
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Ultrasound molecular imaging with molecular-targeted bubbles enables the noninvasive visualization of molecular dynamics in situ. Although some ultrasound contrast agents have been clinically applied for the vascular/Kupffer imaging, however the clinically translatable molecular-targeted bubble has not been developed until now. The aim of this study was to examine the feasibility of molecular-targeted bubbles preparation based on Sonazoid, a clinically available ultrasound contrast agent in Japan. As Sonazoid is stabilized by a membrane of hydrogenated egg phosphatidylserine (PS), we planned to utilize the PS as a scaffold for attaching IgGs and proteins onto Sonazoid. For detecting PS in Sonazoid, annexin V and lactadherin were utilized. By using biotin-avidin complex formation and annexin X, the attachment of IgG onto the surface of Sonazoid was feasible. However, majority of bubbles were disappeared during the bubbles preparation due to the addition of Ca^{2+} for maintaining the binding between PS and annexin V. On the other hand, lactadherin was superior to annexin V, because Ca^{2+} is unnecessary for the binding between PS and lactadherin. Furthermore, the lactadherin-bearing Sonazoid bubbles have an ability to bind with integrin $\alpha v \beta 3$ -expressing endothelial cells (Figure). Because integrin $\alpha v \beta 3$ is well-known to play a key role in angiogenesis, the lactadherin-bearing Sonazoid might have feasibility as a clinically translatable targeted ultrasound contrast agent for angiogenesis.



Development of molecular targeted-bubbles based on Sonazoid

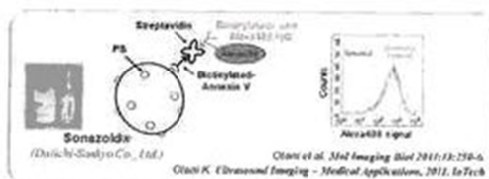


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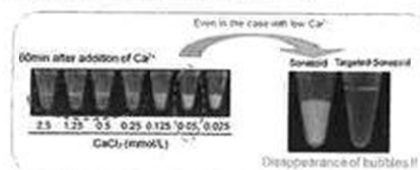
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Background and Aim

- Contrast ultrasound imaging with molecular-targeted bubbles enables the noninvasive visualization of molecular dynamics in situ.
- We reported previously the feasibility of antibody-carrying microbubbles preparation based on Sonazoid, which consists of perfluorobutane gas microbubbles stabilized by a membrane of hydrogenated egg phosphatidylserine (PS), by using annexin V and biotin-avidin complex formation.



- However, the necessity of Ca^{2+} for achieving the binding between PS and annexin V markedly reduced the number of bubbles due to the significant aggregation.



- Additionally, the usage of biotin-avidin complex formation is a barrier for the clinical translation of molecular targeted-bubbles, because of the immunogenicity of streptavidin.

Milk fat globule EGF factor 8 (MFG-E8, lactadherin)

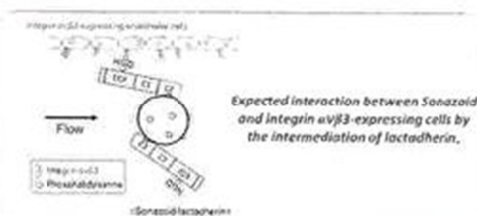
Integrin $\alpha v \beta 3$ or $\alpha v \beta 5$

Stobbs, et al. *PLoS* 1999;27:5417-21
Hirayama et al. *Nature* 2002;417:162-7



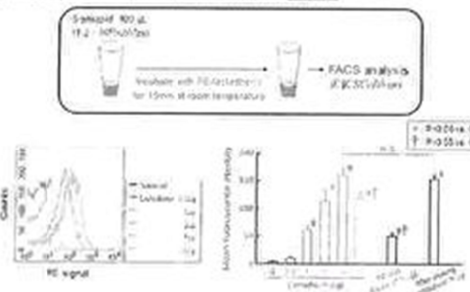
- is a protein secreted from macrophages.
 - accelerates the engorgement of apoptotic cells.
- Binding between PS and lactadherin is Ca^{2+} -independent.

- So, we hypothesized that lactadherin has the potential to be a mediator between PS-containing bubbles and integrin $\alpha v \beta 3$ -expressing cells.
- The aim of this study was to examine whether microbubbles targeted to integrin $\alpha v \beta 3$ could be produced by conjugating Sonazoid with lactadherin.

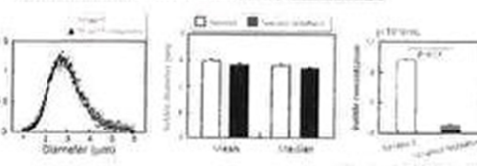


Methods and Results

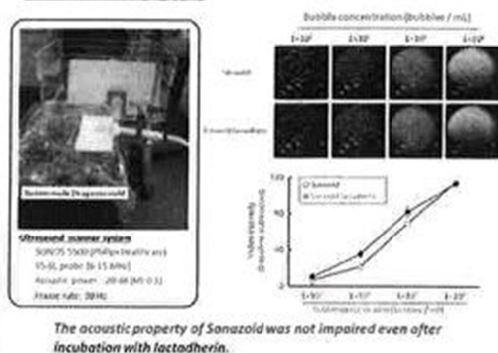
1) Binding between Sonazoid and lactadherin



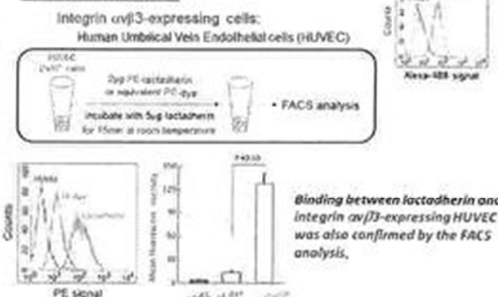
2) Size distribution and concentration of Sonazoid before and after conjugation with 5µg lactadherin



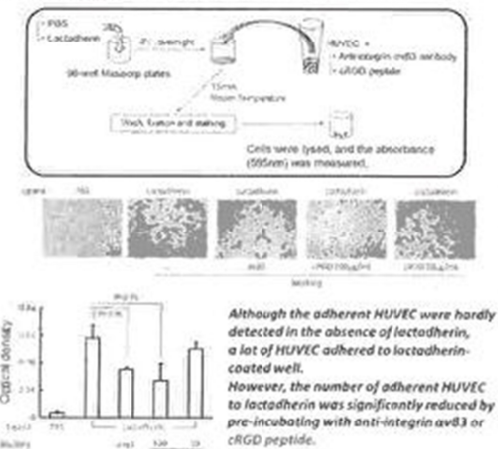
3) Acoustic property of Sonazoid with or without lactadherin conjugation



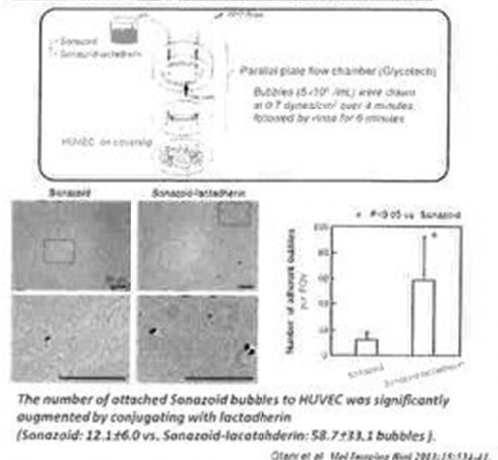
4) Binding between integrin $\alpha v \beta 3$ -expressing cells and lactadherin



5) Specificity of binding between lactadherin and HUVEC



6) Attachment of Sonazoid with HUVEC under shear flow



Summary and Conclusion

- Bubbles size of Sonazoid was not altered even after conjugating with lactadherin.
- Attachment of Sonazoid to integrin $\alpha v \beta 3$ -expressing cells were augmented by the intermediation of lactadherin.
- Because integrin $\alpha v \beta 3$ is well known to play a key role in angiogenesis, the lactadherin-bearing Sonazoid has feasibility as a clinically translatable targeted ultrasound contrast agent for angiogenesis.

Disclosure information

I have no relationships to disclose.