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Title	Comparison of serum and plasma as a source of blood extracellular vesicles: Increased levels of platelet-derived particles in serum extracellular vesicle fractions alter content profiles from plasma extracellular vesicle fractions
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論　文　内　容　の　要　旨
Synopsis of Thesis

氏　　名 Name	張　暁曼
論文題名 Title	Comparison of serum and plasma as a source of blood extracellular vesicles: Increased levels of platelet-derived particles in serum extracellular vesicle fractions alter content profiles from plasma extracellular vesicle fractions (血液由来細胞外小胞のサンプル源としての血清と血漿の比較:血清細胞外小胞画分における血小板由来粒子の混入により含有タンパク質プロファイルが血漿細胞外小胞画分とは大きく異なる)

論文内容の要旨

〔目　的(Purpose)〕

Extracellular vesicles (EVs) are a group of membrane particles that are released from cells, and carry a variety of proteins, nucleic acids, lipids, and metabolites as their cargoes. They are abundantly present in extracellular fluids, such as blood, cerebrospinal fluid, and urine. Among them, blood-derived EVs have attracted much attention as potential diagnostic biomarkers for human diseases. Although both plasma and serum are utilized as a source of blood EVs, it remains unclear whether, how and to what extent the choice of plasma and serum affects the experimental results and the interpretation of EV studies.

〔方　法(Methods)〕

Blood samples were collected from male C57BL/6J mice and human subjects. Plasma was prepared from EDTA-treated blood, and serum was prepared by incubating blood at room temperature. EV-containing fractions were purified by the ultracentrifugation method. The particle numbers and the diameter of EV fractions were measured by nanoparticle tracking analysis (NTA). The protein contents of EV fractions were analyzed by semi-quantitative liquid chromatography-tandem mass spectroscopy (LC-MS/MS) analysis and Western blot analysis.

〔成　績(Results)〕

NTA measurement of mouse blood revealed that the particle numbers in the EV fraction isolated from serum were ~2-fold larger than those derived from plasma. By contrast, the median diameters showed no significant difference between plasma and serum. These results suggest that serum EV fractions contain more EV particles than plasma, while no difference in particle diameter. LC-MS/MS analysis identified 288 and 421 proteins in the plasma and serum EV fractions, respectively, and 432 proteins in total. Gene ontology (GO) analysis focusing on a group of proteins that are increased in the serum EV fractions clearly showed the substantial enrichment of proteins that are associated with platelets, as the GO terms 'platelet aggregation', 'platelet degranulation' (GO, biological process) and 'blood microparticle' (GO, cellular component) were listed as the top terms. Because it is known that platelets produce microparticles and secrete them into the blood, particularly when they are activated during blood coagulation, serum might contain extracellular particles released from platelets more abundantly than plasma. We analyzed the levels of platelet-associated proteins by Western blot analysis, and found that the levels of platelet-associated proteins such as GPIIb, GPIIIa and PF4 were significantly increased in the serum EV fractions. These results indicate that platelet-associated proteins are abundantly present in serum EV fractions. We also analyzed plasma and serum EV fractions from human subjects. Western blot analysis showed a clear trend of selective increase in platelet-associated proteins, suggesting that platelet proteins are also increased in the EV fractions from human serum.

〔総　括(Conclusion)〕

Our results provide experimental evidence that serum EV fractions contain more EV particles than plasma EV fractions, and that platelet-associated proteins are enriched in serum EV fractions. Because platelets secrete microparticles during blood coagulation, EV fractions isolated from serum may contain additional EVs that are released from platelets, which potentially affecting the results and the interpretation of EVs studies when using serum as a source of blood EVs.

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

申請者の張さんは、血液由来細胞外小胞のサンプル源としての血清と血漿の比較を行った。細胞外小胞(EV)は、細胞から放出される小胞であり、血液、脳脊髄液、尿などの体液中に豊富に存在する。EV内にはさまざまなタンパク質や核酸が含まれており、これらの量的・質的な変動がヒトの疾患の病態バイオマーカーとなる可能性が考えられ、注目されている。血液EVの供給源としては血漿と血清の両方が利用されているが、これまで血漿と血清の違いが研究結果やその解釈にどのような影響を及ぼすかどうかは不明であった。本論文では、マウスやヒトの血漿と血清からEVを精製し、それぞれに含まれるEV内のタンパク質成分を比較した。その結果、血清由来EV中には、血液凝固の過程で放出される血小板由来の小胞が多数混入していることがわかった。

研究発表会では、発表は丁寧で、十分に研究内容を伝えているとの評価を得た。研究内容は、近年注目されている血液由来細胞外小胞を用いたバイオマーカー開発において、極めて重要な知見を提供するものであり、学位の授与に値すると考えられる。