Clinical Chemistry

Posttransplantation Bone Marrow Assessment by Quantifying Hematopoietic Cell–Derived mRNAs in Plasma Exosomes/Microvesicles

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Guest:
Dr. Masato Mitsuhashi is the Chief Scientific Officer at Hitachi Chemical Research Center in Irvine, California.

Bob Barrett: This is the podcast from *Clinical Chemistry.* I'm Bob Barrett.

Recent studies have demonstrated that a variety of cells release exosomes or microvesicles into nearby biological fluids such as blood and saliva.

During the exocytic process various proteins' messenger RNA and micro RNA are included in these exosomes.

In the April 2014 issue of *Clinical Chemistry*, a team of Japanese and US researchers looked into using exosomes and their contents in order to assess bone marrow transplantation procedures.

Dr. Masato Mitsuhashi was corresponding author of that paper and he joins us in today's podcast.

Dr. Mitsuhashi is a pediatrician and the Chief Scientific Officer at Hitachi Chemical Research Center in Irvine, California.

Doctor, what exactly is an exosome and why do you think that they may be important in medicine?

Dr. Masato Mitsuhashi: Exosome or [*unintelligible*] vesicle is a small micro vesicles released from cell from inside such as an endosome in the cytoplasm or cell surface.

These vesicles are so small with diameter around 100 nanometer, invisible by a regular microscope that these are not well characterized until recently.

This exosome contains various biological material originated from mother cells. It is considered as a new clinical material for analysis.

Bob Barrett: What types of clinical specimens contain exosomes?

Dr. Masato Mitsuhashi: Well, any biological fluid is now the target of exosome analysis, such as plasma, serum, urine, breast milk, saliva, cerebral spinal fluid and so on, unlimited list.

Bob Barrett: So what is the general procedure for isolating of exosomes and how is your method unique?

Dr. Masato Mitsuhashi: A good standard method is multiple differential centrifugation with the final step of ultracentrifugation of 100,000 g for one hour or more.

This is not practical as routine diagnostic test due to the lengthy procedure. In order to precipitate exosome under regular centrifugation, some polymer solution is commercially available to make equivalent of big molecule.

This is very accepted in academic research but still they were intensive as diagnostics. The uniqueness of our report is the use of filter membrane which absorb exosome quite nicely with more than 95 efficiency, as shown in figure 1 of our paper.

More conveniently, 96-well formatted filter plate can handle 96 samples simultaneously. So we also show the figure of membrane of that exosome under scanning it through microscope in figure 2.

Bob Barrett: What are the components of the exosomes, and why did you choose to analyze messenger RNA?

Dr. Masato Mitsuhashi: Exosome contains a wide range of biological materials such as proteins, lipids, micro RNA and mRNA. The naked mRNA is extremely fragile and instantly digested by endogenous ribonucleus.

However, messenger RNA is safely protected from such ribonucleus attack because of the encapsulation of an exosome membrane.

Thus the function of many messenger RNA species is well characterized and some of them are cell specific or disease specific.

Thus identification or quantification of such well characterized messenger RNA in exosome will lead us to develop new diagnostic never being explored before.

Another reason, why we choose mRNA is that by using gene amplification technology, we can quantify

mRNA very, very sensitively even from a single copy which is far more sensitive than protein analysis.

The main reason is that, as shown in our 2006 *Clinical Chemistry* paper, we have a similar system of filter plate and oligo DT plate combination for whole blood. We just replaced leukocyte-capture filter plate to exosome-capture filter plate.

Bob Barrett: In your study you have examined patients undergoing bone marrow transplantation. Why did you choose this as a clinical objective?

Dr. Masato Mitsuhashi: Many scientists in both academia and industry are now trying to develop diagnostics using exosome, for many types of cancers, metabolic diseases, inflammation, kidney diseases or Alzheimer diseases and so on.

As stated in the paper, bone marrow cells only exist in the bone marrow and rarely escape to peripheral blood. Thus painful bone marrow aspiration is unavoidable.

This procedure of bone marrow aspiration is the one of the most brutal and painful medical procedures.

Our intent was to depress this bone marrow aspiration or at least reduce the frequency of this procedure by analyzing peripheral blood exosome.

And fortunately, maturation processes of bone marrow cells are well characterized and corresponding mRNA are also identified.

Bone marrow transplantation was chosen as our first clinical target because bone marrow condition has drastically changed from a total eradication to full recovery in a short period of time. This is the ideal clinical target to validate our technology.

Bob Barrett: Well finally doctor, is the plasma exosome messenger RNA analysis described in your paper applicable to a routine clinical diagnostics?

Dr. Masato Mitsuhashi: I think so. That's why we did this research. Confirmation of bone marrow recovery much, much earlier than conventional CBC, such as diagnostic [*unintelligible*].

And however clinical adaptation will come later when this assay becomes equivalent to bone marrow aspiration for many other blood diseases such as

anemia, Thrombocytopenia, leukocytosis, leukemia, myelodysplastic syndrome and so on.

Bob Barrett: Dr. Masato Mitsuhashi is the Chief Scientific Officer at Hitachi Chemical Research Center in Irvine, California and he has been our guest in this podcast on the clinical use of exosomes from *Clinical Chemistry*.

I am Bob Barrett. Thanks for listening.