HTPS and QSAR: Screening Methods and Cutting Edge Sciences in Endocrine Disruptor Issue

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Thank you, chairman. It is an honor to be here again and to talk about HTPS and QSAR. What we are dealing with is mainly a hormonally active agent, which acts through receptors. In that sense you can redefine endocrine disrupting chemicals as those compounds that show "adverse" effects. This idea leads to another way of expression, that is, the "receptor-mediated toxicity."

For this first category of "hormonally active agents", we are trying to screen them out in terms of their receptor-mediated hormonal activity. On the other hand, in the second category, or the "definitive testing" methods, we are trying to sort out the receptor-mediated toxicity. Today's talk is about the first category.

In the textbook of endocrinology, you easily find this kind of illustration showing the basic mechanism of nuclear receptor-mediated responses. For the screening purpose, several checkpoints can be utilized. One is the ligand binding process, and the competitive receptor binding assay is the classic one for this. The other is the transcription and translation stages.

Today, we have two speakers about *in silico* modeling of this binding process. For the transcription/translation stage, one method is the reporter assay, which we have Dr. Takeyoshi. About the target organ response using intact or treated animals, the previous session has already covered in detail.

This is the scheme we are considering. I think the basic idea of this scheme is already became universal, because this sort of idea was first expressed be the 1998 U.S. EPA/EDSTAC report.

The *in silico* virtual screening comes as a first step of making a prioritized chemical list for submission to the definitive testing. Then, this *in vitro* high-throughput reporter assay as a second step. Third comes the *in vivo* assay like the uterotrophic assay.

We have two speakers for *in silico* screening. Dr. Weida Tong from the United States will talk about the CoMFA. It is originally good for a system in which you do not know the structure of the receptor itself. This is basically a ligand structure based approach, but now I believe that the structure knowledge of the receptor is incorporated so that the system has gained more accuracy.

From Japan, Dr. Nobuo Tomioka will present the docking model, which, from the beginning, needs the receptor structure knowledge. I would hope that in the near future these systems can somehow predict the position of helix 12, so that the effects on coactivator or cofactor interaction can be predicted.

Hela cell based high-throughput screening robotics system will be presented by Dr. Takeyoshi.

In this session, I would like to introduce a kind of a longitudinal method, which looks into those parts more in detail. One is about transcription and another is about DNA/receptor interaction. For estrogen, it is ERE-ER interaction, and this is done in our lab using this BIACORE machine.

Another speaker we have today is Dr. Julian Hall from Dr. Donald McDonnell's lab. She is talking about cofactor receptor interaction, especially about this LxxLL peptide sequence. For the translation-post-transformational level we have functional genomics from Dr. Bruce Blumberg.

These two, I think, cover this longitudinal path, bridging all these tiers, especially about the toxicological genomics. It has the potential to cover the definitive testing part.

The longitudinal method can be a mechanism-based approach, which is possibly applicable to high-throughput screening and can be connected to definitive testing in the near future.

About surface plasmon resonance data, I would like to briefly show our results. This is what the machine does. It has a sensor tip, and on that tip surface, you can stick any kind of molecule.

In this case, we put the ERE DNA fragment. In the flow, we put estrogen receptor molecule with various ligand molecules. This is the real-time binding and dissociation to this ERE surfaced tip. This is the E2 response which binds fast and when you switch the flow to buffer alone, then the complex dissociates fast.

In contrast, if you do this with ICI compound or tamoxifen, these two anti-estrogens tend to bind much faster, but does not get released from DNA. This system represents a more detailed mechanism of what is going on at the ERE level.

On the sensor tip surface some small peptide can be fixed. In this case we put a LxxLL sequence on the surface and did a similar experiment. Again, this is the ERE response to ICI compound or tamoxifen here. It binds fast and does not get released. If we apply the same sample to LxxLL tip, only the agonist sample results in binding and the antagonist induces no response. Again, in this very artificial cell-free system we may be able to distinguish this kind of effect which we normally need some living cells to monitor.

In the end what I would like to say is that the development of testing methods are getting faster: a new science once got consensus, will soon be applied to test methods. Some of such new science will surely turn out to be very useful and will immediately affect our scheme soon. Well, I think this is it for my introductory talk, and let’s start the session. Thank you.

Q&A

Kavlock : Any questions for Dr. Kanno?

Q : The receptor you used was the human estrogen receptor in the Biocore?

Kanno : Yes, in the Biocore, ERα of human form.

Q : Have you tried other species?

Kanno : Not yet.

Kavlock : Thank you.