Fetal Transcript Profiles for Endocrine Disrupters

George P. Daston

The Procter & Gamble Company

(first slide)

Ladies and Gentlemen, this evening I would like to tell you briefly about my laboratory's research to identify transcript profiles in fetal tissues for endocrine disrupters. It is generally recognized that development is the lifestage that is the most sensitive to endocrine disrupters, yet none of the screens that are being developed use mammalian embryos or fetuses because the manifestations of endocrine-mediated developmental effects tend to be latent. However, it is likely that there are immediate and persistent changes in gene expression in response to hormonally active substances that can be measured in the fetus. Genomic tools will make it possible to identify these characteristic responses.

(second slide)

Our objective is to use genomic data to identify transcript profiles specific to estrogens, anti-androgens or thyrotoxicants, then to develop an inexpensive array-based assay in which the array contains the transcript profiles. It is our hope that the in-life portion of the assay can be made part of the traditional developmental toxicity assay, thereby further saving costs and animals. Because of the limited time for this talk, I will only tell you about our work on estrogens.

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Our hypothesis is that the hormonally active agents of interest will produce immediate and persistent changes in gene expression that can be measured in responsive tissues of the fetus. Support for the hypothesis comes from the knowledge that gene expression is directly controlled by steroid hormones. However, to date we know of relatively few estrogen-, androgen-, or thyroid hormone-controlled genes in the developing mammal.

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To address this lack of knowledge, we have applied genomic methods to screen about 8000 genes of the rat genome, which includes all 7000 genes of known function. We use Affymetrix gene chips.

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Our experimental design involves dosing rats on a daily basis with the test compound from gestation day 11-20. The beginning of the dosing period is just before the rudiments of the reproductive system appear. The end of the dosing period on day 20 is typically when developmental toxicity studies end. Fetal uteri and ovaries are removed on day 20, and samples pooled within litter for mRNA extraction.

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We used three dose levels each of a strong estrogen, ethinyl estradiol, a moderate estrogen, genistein, and a weak estrogen, bisphenol A. The highest dose level of each chemical has been reported in the literature to produce a comparable uterotrophic response in immature rats.

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This is a fetal rat uterus, photographed on an American coin that is about the same size as a one yen coin. We can extract enough mRNA from four uteri to cover a gene chip. The custom array that we

will design specifically for estrogen receptors may be small enough that the mRNA from a single fetal uterus will be sufficient.

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Each compound produced changes in about 600 of the 8000 genes on the chip. Of those, more than 60 show a strong, dose-related response that is consistent among the three compounds. We consider this group of 60 genes to be the transcript profile for estrogens in the fetal uterus and ovaries.

(ninth slide)

This is a partial list of the transcript profile that is upregulated by estrogens.

(tenth slide)

This is a partial list of the downregulated genes of the profile.

(eleventh slide)

Note that the profile includes some genes, like progesterone receptor, that were already known to be estrogen-responsive. It also contains genes for steroid synthesis, cell-cell interaction proteins, growth factors and receptors, and other proteins. There are a number of genes that have not previously been associated with estrogen action.

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There is good correspondence in the magnitude of gene expression change between compounds when the dosages are adjusted for estrogenic potency.

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This is just to provide data that we have confirmed the gene chip results with an alternate method, quantitative RT-PCR. These results are for intestinal calcium-binding protein, a gene already known to be regulated by estrogens.

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Our results lead us to believe that genomic approaches are useful as a way of identifying transcript profiles that are characteristic of hormonally-mediated actions. They are also likely to provide mechanistic insights.

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However, we believe that the use of these profiles should be limited to screening. They are not recommended for hazard assessment or regulation of chemicals because gene expression by itself is not necessarily adverse, and the quantitative relationship between gene expression changes and toxicity is not known.

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In conclusion, we have identified a characteristic transcript profile in fetal tissues for estrogens, using a study design compatible with the traditional developmental toxicity study. Transcript profiling appears to be a promising approach for endocrine disrupter screening, but not risk assessment.

Q&A

Blumberg: We have time for a few questions or comments.

Q: OK, George I am going to throw the same question back at you: did you do any promoter analysis to determine whether all of those genes have estrogen response elements within their regulatory region?

Daston: Yes, we are in the process of doing that — it's a lot of blast searches. Many of them appear to, but not all. If I were going to give you a number I would say less than half of the ones that we have identified have an obvious estrogen response element.

Blumberg: Other questions?
OK, if there are no further questions I would like to thank all the speakers.