In vitro Predictive Toxicology for Breast Cancer

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Ruthann A Rudel[1], Janet M Ackerman[1], Chris D Vulpe[2]
[1]Silent Spring Institute, Newton, MA; [2]University of California, Berkeley

Background

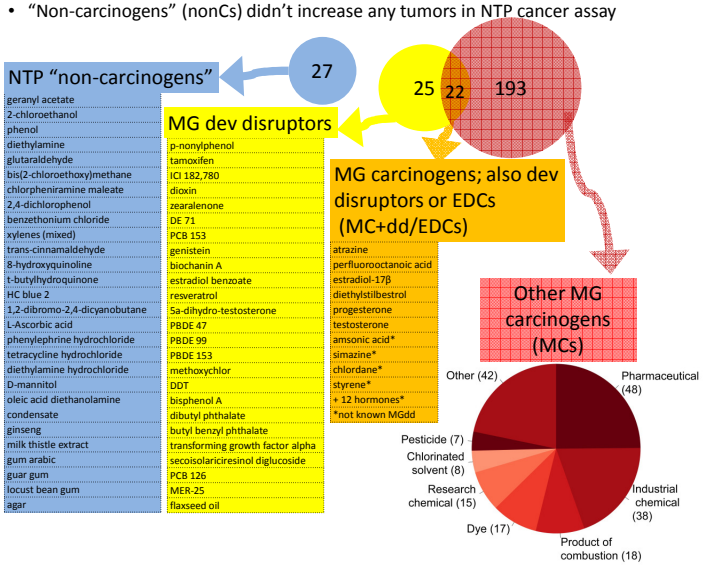
- **Identifying chemicals that increase breast cancer risk could help reduce incidence**
- **High throughput (e.g. ToxCast, Tox21) and computational methods can help predict adverse effects**
- **We are collaborating with EPA and others to develop *in vitro* methods to predict chemicals that increase breast cancer risk**
- **Pathways include genotoxicity, hormone disruption**

Objectives

- **Characterize genotoxicity and nuclear receptor activity of mammary carcinogens and non-carcinogens using publicly-available data**
- **Assess the effects of metabolic activation on test results**
- **ID MCs that act through non-genotoxic mechanisms**

Chemicals of interest

- MG carcinogens (MCs) caused an increase mammary tumors in at least one animal study (Rudel et al. 2007)
- MG development disruptors (MGdds) induced changes in MG development after early life exposure (Rudel et al. 2011)
- "Non-carcinogens" (nonCs) didn't increase any tumors in NTP cancer assay

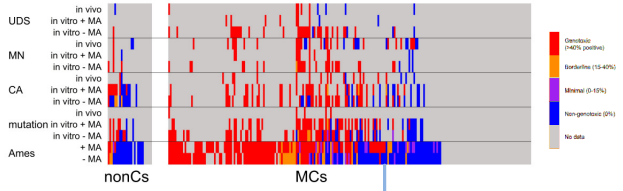


Genotoxicity

NLM's Chemical Carcinogenicity Research Information System

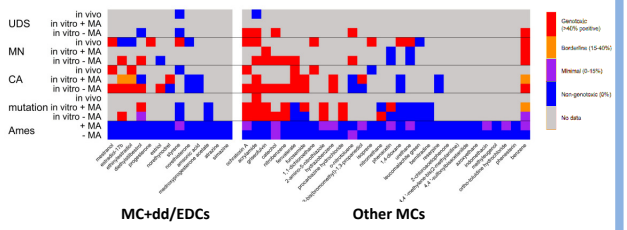
- "mutagenicity" tests: Ames + mammalian mutation, chromosomal aberration, micronuclei, unscheduled DNA synthesis, +/- metabolic activation
- Data available for 156 MCs, 22 nonCs †
- Varied sources, not comprehensive

Genotoxicity of mammary carcinogens and non-carcinogens



- 90% of MCs active (>15% of entries positive) in some study type
- 8% of MCs consistently negative (20% consistently negative *in vitro* w/o metabolic activation)
- 54% of nonCs active in some study type
- 46% of nonCs consistently negative

Mammary carcinogens that are inactive in Ames test



Relying only on Ames to predict MCs would lead to many false negatives - 25% of MCs inactive or minimally active (<15%) in Ames
Why?

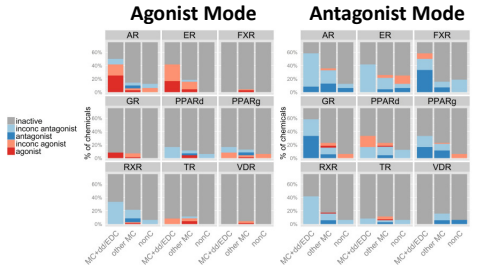
- Ames insensitive to some important genotox mechanisms (e.g. acrylamide,benzene)
- Non-genotoxic mechanisms (e.g. hormones)

†all percentages reported are % of chemicals with data

NR activity

Tox21 (Huang et al. 2011) qHTS beta-lactamase reporter gene assays

- Data available for 82 MCs, 16 nonCs †
- Variable assay reproducibility - ARantagonist and RXRagonist weakest



- In most tests, MCs that are also EDCs/development disruptors were more likely to be active than other MCs, which were more likely to be active then nonCs
- e.g ER agonist assay: 42% of [MC+dd/EDC]s showed agonist activity, compared to 0% nonCs, 8% other MCs

Conclusions

- **Majority of mammary carcinogens are genotoxic in bacterial and mammalian cells**
- **At least 12% of MCs require *in vitro* metabolic activation or *in vivo* testing to show genotoxic activity – lack of MA is an important limitation of high throughput screening so far**
- **Some MCs likely act through multiple mechanisms (genotox and NR-mediated)**
- **MCs are more likely to show NR-activity than nonCs**

Acknowledgments: This work was supported by the California Breast Cancer Research Program.

References

Rudel RA, Attfield KR, Schifano JN, Brody JG. 2007. Chemicals causing mammary gland tumors in animals signal new directions for epidemiology, chemicals testing, and risk assessment for breast cancer prevention. Cancer 109(12 Suppl): 2635-2666.
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SILENT SPRING INSTITUTE
RESEARCHING THE ENVIRONMENT AND WOMEN'S HEALTH

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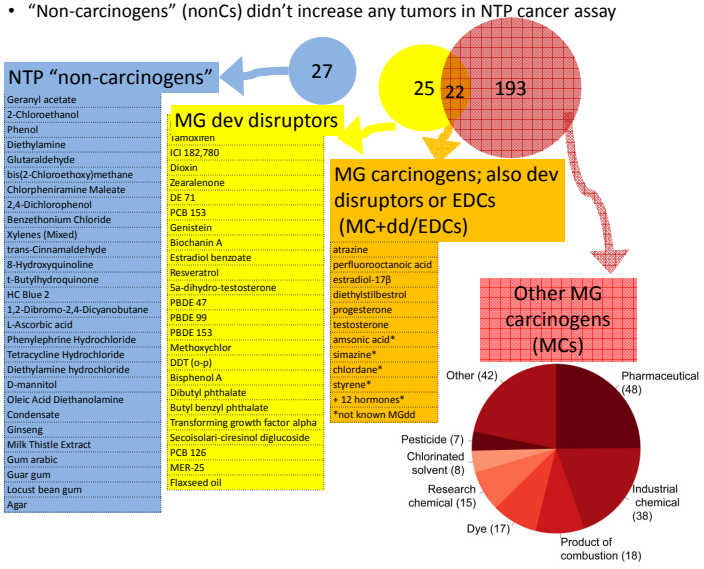

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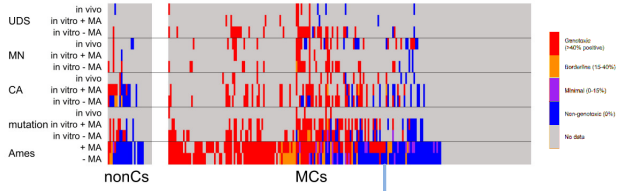

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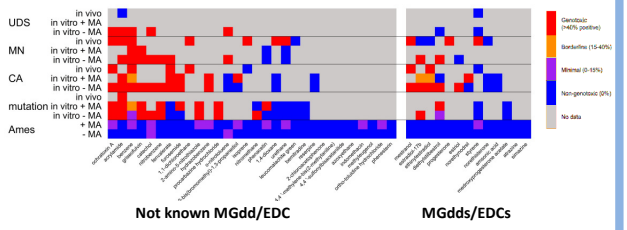

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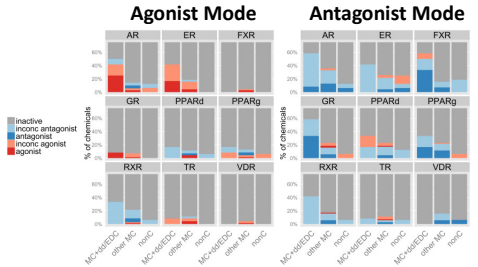

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