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Ovarian Tumors

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Hormonally-Induced Reproductive Tumors: Relevance of Rodent Bioassays (May 22-24, 2006)

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1. Is there sufficient evidence to conclude that these tumors of the reproductive system in humans and experimental animals can result from an altered endocrine milieu (i.e., steroid and pituitary hormones)?

- We cannot conclude that epithelial ovarian tumors are the result of an altered endocrine environment. However, the evidence is suggestive because of the following:
  - Increased incidence around the time of menopause
    - Hormonal changes: Overall decrease in ovarian hormones, increase in LH and FSH. Irregular cyclicity during peri-menopause may contribute.
  - Association with ovulation
    - Studies in nuns showed increased incidence of ovarian cancer
    - Mechanical (disruption of the surface epithelium during follicular rupture) vs. hormonal?
  - Oral contraceptives reduce risk (eg. protection is greater closer to menopause)
  - Epidemiological data suggest estrogen-only replacement therapy may increase risk
  - Increase in numbers of live births reduces risk proportionately
    - Evidence supports that progesterone may provide a protective effect

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1. Is there sufficient evidence to conclude that these tumors of the reproductive system in humans and experimental animals can result from an altered endocrine milieu (i.e., steroid and pituitary hormones)?

- Clear association with elevated gonadotropins and stromal tumor development in experimental rodents
  - Over-expression of LH-beta in transgenic mice
  - Inhibin-alpha knock-out
  - ER-alpha knock-out
  - Numerous genetic mutations that cause follicle loss and associated increase in gonadotropins
  - Studies of chemicals that destroy follicles and reduce steroidogenesis
    - NTP studies with multiple compounds have shown induction of stromal cell tumors, however the mechanisms are unknown/unclear
- Germ cell tumors are not considered associated with endocrine alterations

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1a. Are tumor characteristics and the diagnostic criteria for tumor identification the same between rodents and humans? If not, what are the differences?

- Tubulostromal tumors in mice are distinctly different from stromal tumors in humans.
  - However, morphologically the initial stages of the development of the tubulostromal tumors (formation of epithelial invaginations into the stroma) are similar between mice and humans; the mechanisms of induction of these changes may be different.
- Granulosa cell tumors in rodents and women are similar.
- Spontaneous epithelial cell tumors in rodents are uncommon.
  - Epithelial cell tumors generated in experimental mice can resemble endometrioid or serous human ovarian cancers and mesotheliomas.
- Normal ovarian surface epithelium in rodents and humans, in general, are morphologically similar, although rodents do not develop inclusion cysts.
  - There is a gap in the understanding of the physiology and biochemistry of human and rodent ovarian tumorigenesis and of the molecular events underlying cell transformation.
  - Rodent ovaries have a bursa whereas humans do not; less stroma in rodents than in humans during reproductive years; acyclicity associated with follicle depletion in humans, but not in rodents.

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2. How useful are rodent models for predicting hormonally-induced reproductive tumors in humans?

At this time the current rodent bioassays have not been useful for ovarian cancer. Spontaneous and non-mutagenic chemically-induced rodent ovarian tumors are generally benign.

- Stromal tumors are not predictive because the modest increase in stromal tumors in mice is not indicated in humans (ie. nitrofurantoin).
- For epithelial tumors - None of the recently developed rodent models for epithelial tumors have been tested for predictivity.
  - *in vivo* models
    - No compounds tested have induced epithelial tumors in rodents (except DMBA)
    - The following transgenic models could have potential for testing
      - P53 and Rb conditional knockout resulting in serous adenocarcinomas
      - Activation of K-ras and loss of PTEN leading to endometriosis and endometrioid ovarian cancers
      - MISIIR-TAg transgenic mice developing poorly differentiated adenocarcinomas
  - *in vitro* models of repeated passage of ovarian surface epithelial cells from mice and rats can lead to spontaneous transformation, but have not been tested for predictivity
    - Human ovarian surface epithelial cells senesce *in vitro* after several passages, whereas rodent cells tend not to senesce
- The above and alternative rodent models should be developed and validated.

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2a. What pathological and physiological changes observed in rodent bioassays are assumed relevant for human predictions?

- Chemically-induced ovarian atrophy, as defined by the NTP as reduced follicles, increased interstitial cells, and proliferation of surface epithelium, is a good predictor of ovarian failure, but is a poor predictor of ovarian cancer
- Although there are no known physiological changes that are predictive of ovarian tumorigenesis, elevated gonadotropins and loss of ovarian hormones should be investigated because a majority of ovarian cancers occur in the peri- or post-menopausal period
- This underscores the need for predictive rodent models

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2b. Are there any pre-neoplastic (e.g., hyperplasia) events observed in rodents that are considered predictive of human response?

- Loss of contact inhibition and polarity of surface epithelial cells are observed during early stages of transformation in rodents and possibly in humans.
- Increased stratification of the surface epithelium *may* be a predictor, but there is not sufficient evidence that it necessarily leads to malignancy.
- There are no known molecular markers, but could evaluate: loss of p53, loss of PTEN, over-expression of phospho-AKT in ovarian epithelial cells in prechronic studies.
- Because chromosomal abnormalities are found in in vitro transformed mouse epithelial ovarian cancer cells and human ovarian cancers, these should be investigated in rodent models.
- The profound lack of understanding of the pathogenesis of ovarian tumors in women precludes identification of predictive markers in rodent bioassays at this time.

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3. What do we know of the proposed modes of action for the induction of these tumors in rodents or humans?

Stromal/Granulosa Cells

- Chemical destruction of follicles and subsequent loss of ovarian hormones leads to elevated gonadotropins in rodents
- Elevated gonadotropins induce stromal and granulosa cell tumors in rodents; but there is evidence that chronic elevation of gonadotropins may not be associated with an increased incidence of ovarian tumors in humans. (For example, PCOS patients)

Epithelial Cells

- Gonadotropins (FSH and LH) *in vitro* have been shown in human epithelial ovarian cancer cell lines to stimulate proliferation and invasion. However, elevated gonadotropins in mice do not usually lead to epithelial cell tumors
- Epidemiological studies suggest that incessant ovulation in women is a risk factor for epithelial ovarian cancer. At this time, current rodent models do not support this hypothesis

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3a. Are there key events in the mode of action for hormonal tumors in general, or are they specific for each tumor type? If so, what are the common modes of action?

- Stated previously (Question 3)
  - Chemical destruction of follicles and subsequent loss of ovarian hormones leads to elevated gonadotropins in rodents
  - Elevated gonadotropins induce stromal and granulosa cell tumors in rodents; but there is evidence that chronic elevation of gonadotropins may not be associated with an increased incidence of ovarian tumors in humans. (For example, PCOS patients)
- Sufficient information about the mode of action for epithelial cell tumorigenesis is not currently available, with the exception of mutations in BRCA1 and BRCA2, MSH 1-5, CHK2, and p53 genes
- Epigenetic modification of the promoter of the BRCA1 gene has been observed in sporadic epithelial ovarian cancer

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4. Exposure in the standard NTP rodent cancer bioassays typically commences with young adult animals. Are there any specific modes of action, or tumor types, for which an *in utero* exposure component should be the default experimental paradigm?

- Currently, there is no indication that testing all chemicals in the *in utero* rodent bioassay would be beneficial for identifying increased risk for ovarian tumors.
- Since *in utero* exposure to DES in women and mice induces ovarian cysts, there may be a basis for investigating *in utero* exposure to hormonally active compounds with long-term follow-up of ovarian morphology.
- Exposure to oral contraceptives in younger vs. older women has differential benefits to reducing ovarian cancer risk; therefore, there is some rationale to comparing exposure in juvenile vs. adult animals (or in rodents induced to model peri- and post-menopause).

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4a. How would we best design such studies? (time permitting)

- Not applicable

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5. The default approach for most cancer risk assessments is to assume linearity at low dose-response. Is this appropriate for these modes of action and tumor types?

- Since understanding of the mode of action(s) for ovarian cancer is inadequate, this question cannot be addressed, except in the case of stromal and granulosa cell tumors in rodents resulting from hormonal imbalances (decreased ovarian hormones, increased pituitary gonadotropic hormones). This mode of action would be consistent with a non-linear process at lower doses.

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5a. If not, what evidence would be required to move away from the default approach?

- See Question 5
  - Since understanding of the mode of action(s) for ovarian cancer is inadequate, this question cannot be addressed, except in the case of stromal and granulosa cell tumors in rodents resulting from hormonal imbalances (decreased ovarian hormones, increased pituitary gonadotropic hormones). This mode of action would be consistent with a non-linear process at lower doses.

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5b. How do we (or should we) incorporate the concept of "additivity to background" when endogenous hormones are present with homeostatic control mechanisms?

- For cancers of the ovary, not enough information is known to address this question
- The development of *in vitro* and *in vivo* rodent models is crucial to improving our knowledge of epithelial ovarian cancer biology