NTP Hormonally-Induced Reproductive Tumors - Relevance of Rodent Bioassays Workshop

The NTP held this workshop on May 22-24, 2006, at the NIEHS in Raleigh, NC as part of an activity of the NTP Roadmap to critically review its testing program and determine whether any refinements are needed in the protocols. Over 100 people from academia, industry, government, and non-profit organizations attended the workshop, including an invited panel of 55 scientists with expertise in endocrinology, cancer biology, reproductive toxicology, statistics, and other related fields.

Objectives. The workshop's objectives was to determine the adequacy and relevance to human disease outcome of rodent models currently in use for four types of hormonally-induced reproductive tumors (ovary, mammary gland, prostate, and testis

Format. The workshop opened with a series of presentations on each of the target tissues from the clinical and epidemiological, mode of action, and rodent model perspectives. In order to address the workshop objectives, the invited panel also met in tumor site-specific breakout groups and summarized their discussions in plenary session. The workshop agenda, presentations, background materials, roster of the invited panel and other attendees, and public comments can be found on the NTP website (*http://ntp.niehs.nih.gov* see "Meetings & Workshops" or directly at *http://ntp.niehs.nih.gov/go/18592*).

Findings from the workshop were presented to the NTP Board of Scientific Counselors at its meeting on June 13, 2006. The following text summarizes major points of the workshop discussion. NTP will discuss the findings from this and other Roadmap workshops in October this year to prepare an overall "next steps" strategy.

Workshop Discussions

Testis: The NTP selected testicular tumors for evaluation because the incidence of the disease is increasing in men in certain regions of the world and the current NTP models (F344/N rat and B6C3F1/N mouse) are not optimal for identifying potential human testicular carcinogens. More than 90% of testicular tumors in men are germ cell tumors and only ~ 2% are interstitial or Leydig cell tumors. Germ cell tumors of the testis are the most common tumor type in young men. In contrast, germ cell tumors are only rarely observed in rodents while spontaneous Leydig cell tumors are extremely common in the F344/N rat. The high background incidence of Leydig cell adenomas in the F344/N rat (~70 – 90%) makes it extremely difficult to detect a statistically significant increase in Leydig cell tumors in treated animals. The B6C3F1/N mouse has a very low incidence of spontaneous Leydig cell tumors (< 1%) but also appears to be resistant to developing chemically-induced testicular tumors as none have been identified in NTP studies. Leydig cell tumors in other strains of mice can be chemically induced (particularly by estrogenic agents), but mice are, in general, resistant to the wide variety of agents known to induce these tumors in rats (particularly the Sprague-Dawley and Wistar).

Despite differences in tumor incidence and hormone response, the rodent models are still considered useful. Germ cell tumors are too rare in rodents to evaluate the similarities between rodents and men. The group recommended exploration of the 129 mouse strain which is predisposed to developing male germ cell tumors as a possible model for infantile germ cell tumors in men.

Transformed gonocytes (fetal germ cells) or carcinoma *in situ* (CIS) have both stem- and germ-cell properties and originate from undifferentiated gonocytes, due to poor function of Sertoli and/or Leydig cells during testis development. CIS is considered to be part of the testicular dysgenesis syndrome where environmental and genetic factors can alter Sertoli cell function leading to reduced semen quality or a decrease in Leydig cell function leading to hypospadias and cryptorchidism. For this reason, some members of the group suggested that Leydig cell nodules/hyperplasia in the rat may serve as a surrogate marker for germ cell tumors in men. The breakout group also emphasized that testicular cancer is a reproductive disease. Men with the disease are more likely to also have cryptorchidism and low sperm counts. For this reason, the group suggested that similar outcomes in rodents might serve as early predictors of testicular germ cell tumor induction.

Mammary Gland

Mammary gland tumors were chosen for evaluation in the workshop because breast cancer is the most common type of cancer in women, affecting approximately 1 in 7 women. Another reason for selecting this target site is the concern that the current rodent models used by the NTP (the F344/N rat and B6C3F1/N mouse) are not ideal for identifying potential human breast carcinogens.

There are both similarities and differences between rodents and humans in terms of tumor characteristics and diagnostic criteria. Fibroadenoma is the predominant lesion in some strains of rat but is not considered a pre-malignant lesion in humans. Adenoma and carcinoma in rats are considered to be relevant to breast cancer in humans, although human metastases would be difficult to model as mammary gland carcinomas in rodents rarely metastasize. Premalignant lesions (e.g., atypical hyperplasia) can be observed in both rats and humans. In contrast, pre-malignant lesions in the mouse do not parallel pathological changes in the human. Historical control incidences of mammary gland tumors are lower in female mice compared to rats. In fact, the high background incidence of mammary gland tumors, in particular fibroadenomas (~ 40-45%), in female F344/N rats complicates interpretation of the NTP bioassay. Even though fibroadenomas are clinically relevant they are a benign tumor and may not be predictive of carcinoma in women.

The rat is considered a better model than the mouse for the purpose of identifying potential human carcinogens because mice are generally more resistant to developing chemically-induced mammary gland tumors. There also are endocrine differences between rodents and women that can complicate the interpretation of a positive bioassay finding. Prolactin is the predominant driver of tumor development in F344 rats and it is not clear whether this hormone is a causative agent in humans. The breakout group noted that premature ovarian failure caused by genotoxic agents could produce a false negative response as a loss of ovarian hormones and function results in mammary tissue regression.

In the absence of an ideal model, the existing models are suitable for identifying the potential ability of a compound to induce biological change. This serves as a useful screening function to identify potential carcinogens. However, without a better understanding of the underlying biology and the relevance of the changes in rodents to changes in humans, the full predictive value of these models is not known.

Furthermore, the standard bioassay may miss important opportunities to identify compounds capable of causing breast cancer. Designs that address relevant exposure windows (e.g. *in utero* exposures, exposures during puberty, exposures before a first full term pregnancy) and that distinguish between pre- and post-menopausal risk may be required to detect more agents of potential human concern.

Prostate:
Prostate tumors were chosen for the workshop because prostate cancer is the most common cancer in males in the United States and the third most common cancer world-wide. In addition, this tumor site was selected because it is clear that current conventional rodent models are not useful for identifying prostate carcinogens. Unlike the human condition, spontaneous prostate cancer is very rare in rodents, including the F344/N rat and B6C3F1/N mouse used by the NTP. In a survey of almost 4550 rats and mice used as controls in NTP inhalation or feed studies only 1 carcinoma and 17 adenomas were detected. No substances have been identified as causing prostate tumors in NTP studies.

In addition to differences in spontaneous tumor incidence, the anatomy of the prostate gland in humans and rodents is considerably different. Most significantly, the rodent prostate is lobular and the human prostate is not. Also, the diagnostic criteria and terminology used to describe prostate histopathology in experimental biology, toxicology, and clinical medicine differ.

Despite the differences in prostate anatomy and pathology between humans and rodents, the breakout group felt that it would be prudent to consider prostatic inflammation and hyperplasia in rodent models of prostate cancer to be relevant for humans. Various transgenic models have been developed to study factors related to the pathogenesis of experimental prostate cancer. These transgenic models, however, are generally driven by molecular mechanisms not highly relevant to human prostate cancer. To potentially improve the ability to detect environmental factors that may contribute to prostate cancer, the NTP is encouraged to consider dosing during the early postnatal period when prostate duct development occurs and to search for rodent strains more sensitive to the development of prostate cancer.

Ovary:

Although the life-time risk of developing ovarian cancer is not high (~ 1.5 %), it is the most lethal cancer of the female reproductive system. The absence of a screening test and lack of knowledge among women of the symptoms of ovarian cancer results in a diagnosis that typically does not occur until the later stages of the disease, when survival drops to 20-30%.

Unfortunately, differences in human and rodent ovarian pathophysiology limit the applicability of conventional rodent models for understanding the causes, progression, and therapeutic

interventions of the disease. Approximately 90% of ovarian cancers in women originate from the surface epithelium. In contrast, granulosa cell tumors are the most common type of rodent ovarian tumor and spontaneous epithelial cell tumors are only rarely noted. The low spontaneous incidence of ovarian tumors in rats and mice may limit the utility of the rodent bioassay for detecting possible human ovarian carcinogens. Historical control incidences for various types of ovarian tumors in the F344/N rat and B6C3F1/N mouse are typically less than 1% . Only 10 chemicals show "some" or "clear" association with ovarian tumors in NTP studies and, of these, none are reported in the rat suggesting that ovarian tumors are not readily induced by chemical exposures.

Despite significant differences in ovarian tumors between humans and rodents, certain ovarian observations in rodent bioassays should be assumed relevant for humans. Findings of elevated gonadotropins and loss of ovarian hormones should be considered relevant because this is similar to the endocrine status of menopausal women when most ovarian cancers occur. Other findings, such as ovarian atrophy, are good predictors of ovarian failure but not ovarian cancer. The breakout group also identified several pre-neoplastic events that should be evaluated for predictiveness of human disease including loss of contact inhibition, stratification of the surface epithelium, loss of p53 and protein-tyrosine phosphatase (PTEN), and over-expression of phospho-AKT in epithelial cells. The group discussed several recently developed transgenic models (i.e., P53 and retinobastoma (Rb) conditional knock-out, K-ras activation, PTEN loss, MISIIR-TAg) that may be useful but have not been evaluated for predictivity.

Major Recommendations:

- Utilize alternative models (i.e., transgenic, *in vitro*, etc.) to develop sensitive models for detecting specific types of tumors

- Include additional endocrine responsive endpoints to prechronic studies

- Discontinue use of the F344/N rat

- Evaluate the importance of developmental programming in hormonally dependent tissues leading to pre-neoplastic events and tumors