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Inherited Susceptibility and Acquired Allelic Imbalance in Rat Mammary Carcinogenesis

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Abstract Individual genetically determined susceptibility to cancer as well as acquired epigenetic and genetic organ specific alterations are important considerations in choosing target populations for chemopreventive trials. These individual epigenetic and genetic alterations can also serve as potential biomarkers for chemoprevention clinical trials. In order to model these potential markers for chemoprevention investigations, we are examining a series of interrelated rat models.

Inbred rats vary in their susceptibility to mammary cancer induction by environmental agents. For example, the WF strain is highly susceptible to chemically induced mammary cancer while the Cop rat is almost completely resistant. The F344 is intermediate in susceptibility to chemically induced mammary cancer. These differential susceptibilities are inherited in a dominant pattern. For example, resistance is due to the inheritance of Mcs gene(s) which likely act by altering the differentiation lineage of mammary epithelial cells.

As tumors form in the mammary glands of these rats, they acquire additional epigenetic and genetic alterations. Epigenetic initiation is a very frequent cellular event following carcinogen exposure which may predispose cells to genetic change including allelic imbalance. For example, following a standard dose of NMU or DMBA over 1% of cells are epigenetically initiated. During the carcinogenesis process, initiated cells may acquire genetic change such as oncogene activation and allelic imbalance. Interestingly, the pattern of allelic imbalance appears to be an inherited trait. For example, a non-random loss of heterozygosity (LOH) in rat chromosome 1 following DMBA only occurs in certain strains, such as Cop rats. Interestingly this change does not occur following initiation by ionizing radiation.

It will thus be important to identify these epigenetic and genetic events which underlie mammary carcinogenesis as well as determine their patterns of inherited predisposition and temporal occurrence. Such knowledge is critical if we are to develop new molecular markers for chemoprevention trials. J. Cell. Biochem. 25S:37–40.

Key words: mammary cancer; cancer genetics; epigenetic; chemoprevention

The disease process of carcinogenesis is potentially preventable by several approaches, including that of chemoprevention. In order to evaluate chemoprevention strategies efficiently, they must be tested in cohorts that carry elevated risk to specific cancers. In addition, the ability to monitor intermediate endpoints that are related to elevated individual risk will further enhance early clinical testing of potential chemopreventive agents. Finally, it is likely that many potential agents may target specific subsets of individuals predisposed to a specific organ site cancer. Markers which could identify such a subset would be useful in optimizing chemopreventive strategies. Not only will markers of increased susceptibility of individuals to specific cancer provide cohorts of elevated risk for evaluation and intermediate markers for follow-up but in the best case scenario they could suggest novel targets for the rationale development of new chemopreventive agents and strategies.

In order to identify and characterize both germ-line and acquired genetic and epigenetic markers for future chemoprevention application, it is important to develop and explore animal models for such markers. In that most chemoprevention clinical trials are directed to specific types of cancer it will be important to develop animal models for many organ-specific cancers which are prevalent in human target populations. Examples of these for U.S. populations include colon, breast, prostate, lung, skin, etc. Here we will describe several rat models in which germ-line and acquired genetic and epi-

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genetic changes associated with mammary carcinogenesis can be evaluated as markers of susceptibility, modulatable intermediate endpoints and possible novel chemopreventive targets.

MODELS OF INHERITED PREDISPOSITION TO MAMMARY CANCER

Rat mammary cancers are an important model for human breast cancer in that they both lack a viral etiology and exhibit a wide range of hormonal responsiveness. Inbred rat strains also vary widely in their susceptibility to both spontaneous and induced mammary cancer. The frequency of "spontaneous" mammary carcinomas is directly correlated with susceptibility to the development of mammotropic pituitary tumors which secrete the mammary mitogenic hormone prolactin [1]. The propensity to develop pituitary tumors is inherited as a complex trait under the control of multiple genes [2].

The ability of the carcinogen DMBA to induce cancer in various strains of rats also differ, however, they do not parallel the susceptibilities to spontaneous mammary cancer. The Wistar Furth (WF) rat is an example of a highly susceptible strain while the F344 rat, which is widely used for environmental testing, is of an intermediate susceptibility while the Copenhagen (Cop) rat is almost completely resistant to chemically induced mammary carcinomas [1]. The pattern of inheritance of this phenotype of susceptibility to chemically induced mammary cancer has been defined. The WF rat carries multiple (likely three) independently segregating dominant genes which are responsible for this increased susceptibility to induced mammary cancer. A single allele of any of these multiple genes is capable of providing for the full phenotype of susceptibility. These susceptibility genes are expressed and are active within the mammary parenchyma [3].

The resistance to both chemically induced and spontaneous mammary cancer in the Cop rat is a dominant trait. Gene(s) controlling this trait produce their product(s) within the mammary epithelial cell and also act within the cell in which they are produced. The resistant phenotype is epistatic to the susceptible phenotype [4].

In contrast to the WF and Cop rat, the F344 rat which has an intermediate susceptibility to chemically induced cancer carries neither the WF susceptibility or the Cop resistance genes [1]. Interestingly, this intermediate susceptibility of the F344 to DMBA induced mammary cancer does not extrapolate to either spontaneous [1] or radiation induced mammary cancer [Kamiya and Gould, unpublished].

Defining the mechanism and genetic basis of increased susceptibility in the WF rat may provide a model of a target cohort for prevention trials. In contrast, a better understanding of the genetics and cellular mechanisms underlying the resistant phenotype in the Cop rat may provide an unique approach to breast cancer chemoprevention. To date, the genetic and mechanistic basis of the resistance phenotype has been more fully studied than has that of the susceptible phenotype.

The resistance of the Cop rat strain to chemically induced mammary cancer is a dominant phenotype controlled by one or more Mcs genes. Mcs-1 has been mapped to the centromeric region of rat chromosome 2 [5]. The possible existence of additional genes which contribute to this phenotype is currently under investigation.

The cellular and molecular mechanism underlying this resistance phenotype is also currently under study. Data thus far obtained suggest the hypothesis that resistance is conferred by altered mammary gland differentiation which is under Mcs control. Specifically, virgin rats of strains (Cop and Wky) which have the Mcs phenotype appear to have their ductal cells raised to a more alveolar state of differentiation in comparison to ductal cells of susceptible strains (WF, F344 and SD) [6]. Since carcinomas arise from a ductal cell and not alveolar cell lineage, such a lineage switch could underlie the resistance of Cop rats to mammary carcinogenesis. If this is correct, it could provide a novel target mechanism for the development of a drug which mimics the activity of the Mcs gene(s) product(s).

ACQUIRED SUSCEPTIBILITY TO MAMMARY CARCINOGENESIS IN THE RAT

Genetic

Spontaneous or induced somatic genetic changes can predispose an individual to both premalignant or malignant organ-specific pathologies. Such a paradigm has clearly been demonstrated for human colon cancer [7]. In contrast, acquired genetic changes in breast parenchyma which predispose humans or other mammals to breast cancer have yet to be identi-

fied and characterized. If identified, such changes could serve as marker(s) for identifying cohorts at increased risk and possibly also as modulatable intermediate biomarkers for chemopreventive trials. Human breast cancers have been studied for specific genetic alterations. In contrast to cancers at other sites, the number of prevalent changes identified to date in specific genes has been limited in breast carcinomas. Approximately 25–30% of breast cancer overexpress the neu oncogene while a slightly large percentage have alterations in p53. Other genetic changes such as overexpression of myc and loss or Rb are found in a lesser fraction of mammary cancers [8]. In contrast to these specific acquired genetic changes, a large fraction of mammary cancers contain allelic imbalances at multiple genomic locales. These include both loss and gain of genetic material which often results in loss of heterozygosity (LOH) or gene amplification. Such alteration may be detected by many methods including fluorescence in situ hybridization and microsatellite marker analysis. While allelic imbalance has been demonstrated in frank malignancy, its potential occurrence in small premalignant clonal lesions may provide a somatic genetic marker of predisposed cohorts for specific chemopreventive strategies. The prevalence of cells with such markers could also serve as modulatable markers for phase II chemoprevention trials. Such markers would be extremely valuable for organ-specific cancers, such as those of the breast, which to date lack validated modulatable intermediate endpoints for chemoprevention trials.

In order to evaluate such a possibility, it is important to seek such potential markers in several organ--specific rodent cancers and ask if they occur early in the carcinogenesis process and whether they are modulatable by chemoprevention. From a methodological viewpoint, it is desirable to maximize assay sensitivity and reproducibility so that multiple genetic alterations in very small populations of cells can be detected. Currently, the best method that meets this requirement is the use of alteration in microsatellite markers to detect allelic imbalances as well as changes in the size of a -specific microsatellite region.

We have begun to characterize induced rat mammary carcinomas for allelic imbalance. Microsatellite markers in the form of dinucleotide repeat markers are found at intervals of less than 30 kb throughout most mammalian genomes including human, mouse and rat. A very large number of these markers have been characterized and mapped in humans and mice. Much effort is currently being invested in mapping such markers in the rat [9]. Currently, sufficient well characterized and mapped markers exist in the rat to assay most of the genome for allelic imbalance with good resolution [9]. Characterizing the genome of the F_1 offspring of two inbred rat strains for allelic imbalance is more straightforward than similar studies in humans in that approximately half of all microsatellite markers are informative in any given F_1 cross [9].

We have thus far characterized both chemically and radiation induced mammary carcinomas for allelic imbalance using microsatellite markers. DNA is extracted from either isolated tumor epithelial cells or whole tumors. Control DNA is isolated from individual spleens. The DNA from these F_1 rats are quantitatively analyzed for alterations at -specific microsatellite locations. For each location, -specific regions of the DNA is PCR amplified using primers -specific for unique sequences flanking the marker of interest. All markers chosen can distinguish microsatellite polymorphic size differences in the alleles from each parent strain. Following PCR amplification, the products are separated on sequencing gels and imaged based on incorporated p32 nucleotides using a phosphoimager. Normally, the ratio from band density of -specific alleles in the tumor should parallel that of spleen DNA. Deviations of greater than 25% are scored as allelic imbalances. The actual imbalance in the tumor DNA is likely larger than that measured due to stromal contamination in the original sample.

Using a large number of markers located throughout the genome, we have analyzed mammary carcinomas induced by chemicals (DMBA, NMU) and ionizing radiation in several different F_1 rat strain crosses. Sporadic alterations in microsatellite markers in tumors but not spleens were frequently found in all groupings of carcinomas tested. Thus far only 1 statistically confirmed non-random imbalance has been detected. This imbalance was detected in a chromosome 1 region centered in the area of marker R1030.

Interestingly, this non-random allelic imbalance was detected in chemically induced but not radiation induced carcinomas. Also, impor-

tantly this imbalance was only found nonrandomly in a subset of genetic F_1 crosses. Initially, it was found that the WF × Cop F_1 but not the WF × F344 cross. This led to the hypothesis that it might be associated with the dominant Mcs phenotype contributed by the Cop parent. This allelic imbalance was also found in the F344 × Cop F_1 cross but not in a F_1 cross of the mammary cancer resistant Wky rat and the F344 rat. Since both Cop and Wky carry the Mcs phenotype under similar genetic control [10], the hypothesis was rejected. The allelic imbalance centered at the R1030 marker while associated with the presence of a Cop allele does not associate with a Mcs phenotype.

This finding suggests that the use of somatic allelic imbalance as a marker of risk may be confounded by unknown modifier genes carried by individual or -specific inbred strains of animals. This genetic interaction must be clarified before such allelic alteration can be reliably used in human trials. In addition, the scarcity of acquired genetic markers in rat mammary carcinomas suggest by extension a possible lack of acquired genetic alteration in premalignant cells in the rat mammary gland. This suggests the need to seek alternative premalignant markers such as epigenetic changes in premalignant cell populations.

Epigenetic Changes

In addition to genetic alterations, the premalignant adenoma of the human colon also possesses epigenetic changes. The best characterization of these is genomic hypomethylation [7] which could potentially alter gene expression. It is likely that epigenetic changes may exist in other organ-specific premalignant lesions such as those in the breast.

Recent evidence has suggested the possibility that the initiation of the carcinogenic event by chemicals and radiation in the rat mammary gland may occur via an epigenetic mechanism [11]. This postulate is based on the finding that the frequency of cellular initiation of carcinogenesis by exposure to a high dose of radiation or the carcinogen NMU is approximately 10^{-2} [11]. In other words, 1 in 100 cells are initiated. The molecular mechanism underlying this very high frequency of initiation is not likely to be a mutagenic event in that such events have estimated frequencies following such carcinogen exposure of several orders of magnitude less than the measured frequency of initiation. Interestingly, progression of the initiated cell is held in check for a period of time by its surrounding parenchyma. If only a few carcinogen exposed cells are transplanted, a high frequency of initiation is quantified. However, the measured frequency of initiation appears to decrease as the graft size is increased. This is due to the larger number of interacting cells at the graft site [11]. This data that initiation is a very high frequency event suggests that the earliest events in the carcinogenic process may be of an epigenetic rather than of a genetic type which would have a much lower frequency. If so, it may be useful to begin to characterize such epigenetic events as both markers for cohorts with increased breast cancer risk as well as modulatable intermediate endpoints for chemoprevention clinical trials. Importantly, such epigenetic initiation events are potentially reversible by chemopreventive intervention.

REFERENCES

1. Gould MN, Wang B, Moore CJ (1989): Modulation of mammary carcinogenesis by enhancer and suppressor genes. In Colburn NH (ed): "Genes and Signal Transduction in Multistage Carcinogenesis." New York: Marcel Dekker, Inc., pp 19–38.
2. Wiklund J, Rutledge J, Gorski J (1981): A genetic model for the inheritance of pituitary tumor susceptibility in F344 rats. Encorinology 109:1708–1714.
3. Gould MN (1986): Inheritance and site of expression of genes controlling susceptibility to mammary cancer in an inbred rat model. Cancer Res 46:1199–1202.
4. Zhang R, Haag JD, Gould MN (1990): Site of expression and biological function of the rat mammary carcinoma suppressor gene. Carcinogenesis 11:1765–1770.
5. Hsu LC, Kennan WS, Shepel LA, Jacob HJ, Szpirer C, Szpirer J, Lander ES, Gould MN (1994): Genetic identification of Mcs-1, a rat mammary carcinoma suppressor gene. Cancer Res 54:2765–2770.
6. Hsu LC, Gould MN (1993): Cloning and characterization of overexpressed genes in the mammary gland of rat strains carrying the mammary carcinoma suppressor (Mcs) gene. Cancer Res 53:5766–5774.
7. Cho KR, Vogelstein B (1992): Genetic alterations in the adenoma-carcinoma sequence. Cancer 70:1727–1731.
8. Gould MN (1993): Cellular and molecular aspects of the multistage progression of mammary carcinogenesis in humans and rats. Sem Cancer Biol 4:161–169.
9. Jacob HJ, Brown DM, Bunker RK, Daly MJ, Dzau VJ, Goodman A, Koike G, Kren V, Kurtz T, Lernmark A, et al. (1995): A genetic linkage map of the laboratory rat, Rattus norvegicus. Nat Genet 9:63–69.
10. Haag JD, Newton MA, Gould MN (1992): Mammary carcinoma suppressor and susceptibility genes in the Wistar-Kyoto rat. Carcinogenesis 13:1933–1935.
11. Kamiya K, Yasukawa-Barnes J, Mitchen JM, Gould MN, Clifton KH (1995): Evidence that carcinogenesis involves an imbalance between epigenetic high-frequency initiation and suppression of promotion. Proc Natl Acad Sci U S A 92:1332–1336.