Reduced mammary gland carcinogenesis in transgenic mice expressing a growth hormone antagonist

M Pollak1, M-J Blouin1, J-C Zhang1 and JJ Kopchick2

1Cancer Prevention Research Unit of the Jewish General Hospital and McGill University, Montreal, Quebec, H3T 1E2, Canada;
2Edison Biotechnology Institute and Department of Biomedical Sciences, Ohio University, Athens, Ohio, 45701, USA

Summary Several reports have provided evidence that body size early in life is positively correlated with risk of subsequent breast cancer, but the biological basis for this relationship is unclear. We examined tumour incidence in transgenic mice expressing a growth hormone (GH) antagonist and in non-transgenic littermates following exposure to dimethylbenz[a]anthracene (DMBA), a well characterized murine mammary gland carcinogen. The transgenic animals had lower IGF-I levels, were smaller in terms of body size and weight, and exhibited decreased tumour incidence relative to controls. The demonstration that both body size early in life and breast cancer incidence are influenced by experimental perturbation of the GH–IGF-I axis in a transgenic model provides evidence that variability between individuals with respect to these hormones underlies the relationship between body size early in life and breast cancer risk observed in epidemiological studies. © 2001 Cancer Research Campaign http://www.bjcancer.com

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Insulin-like growth factor-I (IGF-I) is a peptide with both mitogenic and anti-apoptotic properties. Unlike other growth factors, it has characteristics of both a tissue growth factor and an endocrine hormone (Jones and Clemmons, 1995; Rajaram et al, 1997). Circulating IGF-I levels are subject to complex physiological regulation, and vary considerably between normal individuals (Harrela et al, 1996). Most circulating IGF-I originates in the liver, and growth hormone is a key up-regulator of hepatic IGF-I production. IGF bioactivity in tissues is not merely a function of circulating concentration; local expression of IGFs, IGF-binding proteins, and proteases that digest IGF-binding proteins are also important factors. Clinical and experimental evidence suggesting important roles of IGF physiology in carcinogenesis and neoplastic progression have been the subject of recent reviews (Burroughs et al, 1999; Khandwala et al, 2000; Pollak, 2000).

Several epidemiological studies provide evidence that circulating IGF-I level is related to breast cancer risk among premenopausal women (Peyrat et al, 1993; Bruning et al, 1995; Bohlke et al, 1998; Hankinson et al, 1998; Toniolo et al, 2000; Li et al, 2001). A recent report (Byrne et al, 2000) provides evidence that circulating IGF-I level is highly correlated with mammographic density, which has previously been shown to be related to breast cancer risk. Separate data also suggest a relationship between circulating IGF-I level and risk of other cancers (Baringa, 1998; Chan et al, 1998; Maisron et al, 1998; Wolk et al, 1998; Burroughs et al, 1999; Holly et al, 1999; Manousos et al, 1999; Yu et al, 1999; Holly and Smith, 2000; Shanyfelt et al, 2000). The mechanism underlying the IGF-I-cancer risk relationship remains unclear, but may relate to increased epithelial cell turnover and/or increased cell survival in individuals with higher IGF-I tissue bioactivity (Cohen and Ellwein, 1990; Ng et al, 1997; Pollak et al, 1999; Pollak, 2000).

Separate research has provided evidence that body size or weight early in life is positively correlated with subsequent risk of breast cancer (Michels et al, 1996; Stavola et al, 2000; Kajser et al, 2001). The biological basis for this observation has been the subject of considerable speculation (for example, Vatten, 1996; Stoll, 1997; Signorelllo and Trichopoulos, 1998) but has not been addressed in experimental work. In view of evidence that body size early in life is related to cord blood IGF-I level (Ong et al, 2000), we hypothesized that the reason that there is a relation between body size early in life and subsequent breast cancer risk is that both are influenced by IGF-I levels. IGF-I levels are known to vary substantially between normal individuals, and approximately 50% of this variation is attributable to genetic factors which have not yet been characterized (Harrela et al, 1996). To address this hypothesis, we studied the effect of experimental perturbation of the GH–IGF-I axis in a transgenic mouse model on experimental mammary gland carcinogenesis.

Transgenic mice that constitutively express a bovine (Chen et al, 1990, 1991) or human (Chen et al, 1994) GH antagonist have been produced. In the present study, we examined tumour incidence in GH antagonist transgenic (GHA-tg) mice and in non-transgenic littermates in the standard dimethylbenz[a]anthracene (DMBA) breast carcinogenesis model in order to determine if an intervention targeted specifically at the GH–IGF-I axis would influence breast carcinogenesis.

28 8-week-old female GHA-tg mice (Chen et al, 1990) and 26 control littermates of the same age were given, by gavage, DMBA (70 µg g–1 body weight) resuspended in peanut oil. The gavages were administrated once a week for 6 weeks. After the last gavage, tumour appearance was monitored weekly and tumours were measured with a caliper. Mice with tumours were sacrificed when tumours reached ~1 cm in diameter or 39 weeks after DMBA treatment. The mice were weighed, measured and blood was collected.

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Correspondence to: M Pollak

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Table 1  Characteristics of control and growth hormone antagonist transgenic mice

|                             | Body length (cm) | Body weight (g) | Circulating IGF-I concentration (ng ml⁻¹) | Percentage without tumour at 39 weeks (%) |
|-----------------------------|------------------|-----------------|------------------------------------------|----------------------------------------|
| Control mice                | 9.5 ± 0.5        | 31.0 ± 2.6      | 188.9 ± 8.8                              | 31.6                                   |
| Growth hormone antagonist transgenic mice | 7.6 ± 0.2        | 17.7 ± 3.2      | 104.5 ± 2.6                              | 68.2                                   |
| P value                     | <0.005           | <0.0001         | <0.0001                                  | <0.001                                 |

The results are expressed as the mean ± SEM.

Figure 1  Tumour incidence in growth hormone antagonist (GHA) transgenic mice vs. control mice following DMBA exposure. During the 39 weeks following DMBA treatment, tumour appearance was monitored as described in the text. By cardiac puncture at time of sacrifice. Serum was frozen and subsequently assayed using an ELISA for rodent IGF-I (reagents from Diagnostic System Limited, Webster, Texas). Mice were handled in accordance with a protocol approved by McGill University and Lady Davis Institute animal care committees, and in accordance with the UKCCCR guidelines, and were maintained in a temperature-controlled environment with diurnal cycle of 12 hours of light and 12 hours of darkness, on an ad libitum diet (UKCCCR, 1998). Statistical tests (Student’s t-test) were two-sided; P values of <0.05 were considered to be significant.

The GHA-tg mice had lower levels of circulating IGF-I than control littermates (Table 1). GHA-tg mice were significantly smaller and lighter than non-transgenic animals, as previously reported (Chen et al, 1990). The GHA-tg female mice had grossly normal mammary glands, and normal ductal and stromal architecture on routine microscopy. Lactational performance of these mice was normal.

Tumour incidence was substantially reduced in GHA-tg mice compared to control mice (Table 1, Figure 1). The difference between the tumour incidence curves was highly significant (P <0.001). The tumours were breast adenocarcinomas, as previously reported with the DMBA model (Russo and Russo, 1996).

Epidemiological studies have provided evidence that individuals with circulating IGF-I levels at the high end of the normal range have higher risk for premenopausal breast cancer than individuals with IGF-I levels at the low end of the normal range (Peyrat et al, 1993; Bruning et al, 1995; Bohlke et al, 1998; Hankinson et al, 1998; Toniole et al, 2000; Li et al, 2001). Separate evidence suggests that body size and weight early in life are related to subsequent breast cancer risk (Michels et al, 1996; Stavola et al, 2000; Kaijser et al, 2001). We show in a transgenic model that the phenotype associated with expression of a GH antagonist includes not only reduced body size and reduced circulating IGF-I level, but also reduced susceptibility to DMBA-induced mammary gland carcinogenesis. This suggests that inter-individual variations in the GH–IGF-I axis have important influences on both birthweight and breast cancer risk, and thus may explain at least in part the association between birthweight and breast cancer risk.

Our study design does not allow us to determine if there is a critical time at which IGF-I levels influence risk. It is known that a significant component of the inter-individual variation in IGF-I levels is genetically determined (Harrela et al, 1996), and it is possible that the influence of IGF-I on cancer risk operates through effects on epithelial cell renewal dynamics throughout life, including prenatal life, the period of pubertal breast development, and young adulthood.

The recent description of the safety and IGF-I lowering action of a GH antagonist in acromegalics (Trainer et al, 2000), together with our experimental results, raise the possibility that targeting the GH–IGF-I axis may represent a novel approach for cancer risk reduction for individuals with IGF-I levels at the high end of the normal range. This would be particularly relevant to the challenge of risk reduction for individuals who have known cancer risk factors such as carcinogen exposure, a germ line mutation in a cancer predisposition gene, or mutagen sensitivity, whose risk may be amplified by high IGF-I levels (Wu et al, 2000). It remains to be determined, however, if reduction of IGF-I levels from the high to the low end of the normal range in adulthood would in fact reduce IGF-I related cancer risk. It is conceivable that IGF-I levels in middle age are related to cancer risk only because they represent a surrogate for levels at some early critical period, such as puberty, at which time they influence risk. If this is the case, then interventions later in life will have little effect. Animal models of this issue are difficult due to species differences in growth, development and puberty. Nevertheless, early work involving perturbation of the GH–IGF-I axis with somatostatin analogues subsequent to carcinogen exposure in murine mammary carcinogenesis systems (Pollak and Schally, 1998) does provide evidence for reduction mammary cancer incidence even if treatment is deferred and should provide a foundation for further research.

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