Humoral Hypercalcemia of Malignancy in Female F344 Rats Implanted with a Transplantable Rat Pulmonary Carcinoma Line (IP)

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ABSTRACT. A transplantable rat pulmonary carcinoma line (IP) in F344 rats is a useful animal model for humoral hypercalcemia of malignancy (HHM). The present study analyzed the degree of HHM by implanting IP into F344 female rats aged 6, 20 or 45 weeks. IP-bearing females developed elevated plasma parathyroid hormone-related protein levels, hypercalcemia and increased osteoclastic activity as well as calcification in various organs. The severity of such HHM differed depending on age; particularly, calcification showed age-dependent reduction. HHM development in IP-bearing females may be influenced by age-related factors.

KEY WORDS: age-dependent factor, humoral hypercalcemia of malignancy, pulmonary rat carcinoma.

Humoral hypercalcemia of malignancy (HHM) is a paraneoplastic syndrome that has been often reported in human cancer patients with squamous cell carcinomas and pulmonary carcinomas or in dogs with apocrine gland-derived adenocarcinomas and lymphomas [5, 6]. The HHM is characterized pathobiologically by hypercalcemia, systemic calcification in various organs such as kidneys and lungs, and activated osteoclasts as well as increased level in parathyroid hormone-related protein (PTHrP) [5, 7, 12]. Because of having biological functions similar to parathyroid hormone (PTH), the PTHrP is considered to play a central role in the development of HHM [5, 7, 12]. However, the pathogenesis of HHM is very complicated and remains to be clarified [3, 5, 11].

As an animal model for HHM, we had recently established a transplantable rat pulmonary carcinoma line (IP) in F344 rats [4]. IP-bearing male rats (n=49; in age from 6 to 20 weeks) used in serial transplantation until the 26th passages developed HHM with a 100% tumor take rate [4]. On the contrary, interestingly, the tumor take rate in IP-implanted female rats ranging in age from 6 to 30 weeks was about 65% (15/23). In addition, as compared with HHM in the males, the degrees of HHM in the females varied from case to case. These findings suggest that the development of HHM depends on some factors such as sex and age in implanted hosts. In the present study, thus, we focused on the relationship of age with HHM development in females.

The following different three experiments (Exs I, II and III) were performed. The experiments were in compliance with our institutional guideline for animal care. Female F344 rats (Charles River Japan, Hino) at the age of 6, 20 and 45 weeks were used in Exs I, II and III, respectively. A tissue fragment (2 mm in diameter) of IP was transplanted subcutaneously into the interscapular region through a trocar under ether anesthesia [4]. In each Ex, sham-operated, age-matched females were served as controls. The number of rats used in each Ex and post-implant examination (PE) weeks are shown in Table 1.

Tumor growth was estimated by the formula (a × b²/2 : a, major axis; b, minor axis). Tumors in Exs I and II showed similar growth curve (Fig. 1), whereas those in Ex III slowly grew, with a significant decrease in contrast to tumor vol-

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umes of Exs I and II (Fig. 1); the respective average tumor weight at PE-week 3 in Exs I, II and III was 10.5, 11.3 and 4.3 g. Thus, rats in Ex III were observed until PE-week 4. Body weights of transplanted rats in all Exs tended to be decreased; the mean ± standard error (SE) at PE-week 3 in transplanted vs. control rats was 99.3 ± 2.7 vs. 130.5 ± 1.6 g in Ex I, 143.5 ± 14.4 vs. 173.7 ± 4.7 g in Ex II and 190.7 ± 8.7 vs. 203 ± 3.5 g in Ex III. Particularly, the decreased level was the greatest in Ex I, with a statistical significance at $P < 0.05$ by Student $T$ test. Plasma PTHrP levels, measured by the immunoradiometric assay (IRMA) [4], were increased in IP-bearing rats in all Exs, except one rat in Ex III (Fig. 2), whereas those in all controls were below the detection limit (< 1.1 pM). Serum calcium levels at PE-week 3 in IP-bearing rats of all Exs were significantly elevated at $P < 0.05$; the mean ± SE in implanted vs. control rats in Exs I, II and III was 13.55 ± 0.61 vs. 10.35 ± 0.06, 16.08 ± 1.48 vs. 10.07 ± 0.09, and 13.45 ± 1.81 vs. 10.01 ± 0.06 mg/dl, respectively.

Histologically, calcium deposition, demonstrable by the von Kossa's stain, was observed mainly in the kidneys, lungs and heart of IP-bearing rats examined at PE-weeks 2 and 3 in Exs I and II. In the kidneys, the calcification was seen in the lumina and basement membranes of renal tubules (Figs. 3A, B). In the lungs and heart, the major sites of calcification were the alveolar septa, and in arterial wall and myocardium around the affected arteriae. Interestingly, the severity of calcification in these organs was greater in Ex I than in Ex II, as shown in Table 1 for the kidneys. More interestingly, no calcification was observed in any organs of all rats examined at PE-weeks 3 and 4 in Ex III (Fig. 3C and Table 1). Figure 5 shows the ratio of osteoclastic areas in the femur of implanted rats to those of controls in each Ex. The osteoclastic areas (Fig. 4), stained positively by the tartrate-resistant acid phosphatase (TRAP, a marker enzyme of osteoclast function) method, were measured by an Image Analyzer (Mac Scope, Mitani Inc.) [4]. As shown in Fig. 5, in all Exs, the highest increase was seen in Ex II. No significant pathological findings, except for calcification, were found.

It has been speculated that PTHrP produced excessively by tumor cells stimulate osteoclastic activity, resulting in hypercalcemia and calcification in various organs, although the detailed mechanisms have not yet been decided [5, 7, 11, 12]. We previously demonstrated that IP tumor cells produce PTHrP [4]. In fact, IP-bearing females in all Exs showed elevated plasma PTHrP levels, hypercalcemia and increased osteoclastic activity. However, calcification was not seen in any organs of implanted rats in Ex III, and the degree of calcification in Ex I and II was reduced with advancing age (Table 1). In addition, the ratio of osteoclastic activity (Fig. 5) was the greatest in Ex II; this may imply that PTHrP acts more strongly on the just completed bone of 20 week-old rats than the developing bone of 6 weeks-old rats or the already matured bone of 45 week-old rats. Moreover, tumor growth (Fig. 1) and body weight change were the mildest in Ex III. These findings at least suggested that age-related factors might have influenced the development of HHM in IP-bearing females. Particularly, it was found that the severity of calcification is reduced depending on age, because IP-bearing females in Ex III failed to develop calcification in kidneys, heart and lungs.

Since PTH/PTHrP receptors are present exclusively in the osteoblasts, the osteoblast is a target cell for PTHrP [10]. Thus, the osteoclastic activity in HHM is regulated mainly...
Estrogen plays an important role in controlling bone remodeling and calcium metabolism [1, 2]. It is conceivable that estrogen level and osteoblastic activity may be different depending on age in females (growing, adult or old); such factors might have influenced the age-dependent difference in HHM in IP-bearing females. Our previous study showed that all F344 males implanted with IP developed HHM [4], of which severity appeared to be much greater than that seen in female rats in the present study. There have been no papers reporting age/sex-dependent changes in HHM development. The severity of HHM in human cancer patients is regarded as a crucial determinant for prognosis [9]. IP is a useful animal model that can develop HHM similar closely to that seen in human cancer patients [4]. It is interesting to investigate age/sex-related factors (presumably, bone remodeling balance or sex hormones) by using IP, in addition to pursue the mechanism of tissue calcium deposition.

REFERENCES


