ABSTRACT

SCID mice are a model of human severe combined immunodeficiency disease and are deficient in B cell function in addition to T cell function. Tumors from other species are easily transplanted into SCID mice and will grow without being rejected. We previously reported that the chemokine BRAK/CXCL14 is expressed in normal cells but its expression is down regulated in an in vitro cancer progression model, suggesting that it has the potential for antitumor activity. Here we report that the growth of BRAK/CXCL14 expression vector-transfected oral cancer cells was completely (100%) suppressed in SCID mouse xenografts even though mock-vector introduced control tumor cells grew well with 100% of animals developing tumors. In addition, suppression of xenografts was much faster and the rate was much higher in SCID mice than in T cell function-deficient nude mice. These data indicate the possibility that BRAK expression inhibits tumor cell establishment by regulating interactions between tumor stem cells and NK cells and/or suppressing formation of tumor microvessels.

Tumors develop in multiple steps (6, 19, 25), and tumor progression is dependent on the balance of the expression between tumor progression-promoting and -suppressing genes being in favor of the former at each step (1, 2). In order to prevent tumor progression, many investigators have searched for molecules that are over-expressed during tumor progression as target molecules for therapeutic drugs and have tried to prevent tumor progression by inhibiting these tumor-promoting molecules. However, drugs for many of the target molecules were not successful for clinical applications owing to the serious side effects of these drugs, which is not surprising because these target molecules are also important for normal development and maintenance of tissues and for homeostasis of our body (16, 22).

On the other hand, activation of presumptive tumor suppressor(s) or inhibition of their down-regulation may be much more promising for the prevention of tumor progression without significant side effects, because these molecules are supposedly present abundantly in normal tissues. In the course of our study to find an endogenous tumor suppressor(s) for oral cancers (OC), we searched for molecules down-regulated in OC cells when the cells were treated with epidermal growth factor (EGF), whose receptor is frequently over-activated in OC. The expression of BRAK, which is also known as CXC chemokine ligand 14 (CXCL14), was down-regulated significantly by the treatment of OC cells with EGF as observed by cDNA microarray analysis followed by reverse-transcriptase polymerase chain reaction analysis. In order to investigate whether BRAK/CXCL14 have a tumor-suppressing effect in vivo, we prepared BRAK/CXCL14-expression vector-transfected tongue tumor-derived cells (HSC-3 BRAK) and cloned them. No difference in the growth rates of these cells was observed in vitro
In this study, we injected 3 clones of BRAK-expressing (HSC-3 BRAK) and 10 clones of mock vector-transfected (HSC-3 Mock, $5 \times 10^6 / \text{site}$) cells prepared as described previously (13), into both sides of the back region of 6-weeks old 10 female SCID mice (FOX CHASE SCID, C.B.-17/Scid- scid/ scidNcl; Clea Japan, Tokyo), respectively. The HSC-3 BRAK cells were shown by western blotting to express a 10 times higher amount of BRAK/CXCL14 protein than HSC-3 Mock cells. SCID mice used were confirmed not to be leaky by the determination of immunoglobulin production at age of 6 weeks. Tumor volumes was calculated according to the following formula: $a b^2/2$, where $a$ is the longer and $b$ is the shorter dimension as described (13).

All the xenografts of HSC-3 Mock grew well (Fig. 1 Mock) and formed tumors of appreciable size by 27 days after xenografting (Fig. 2, A and B), as would be expected in SCID mice. On the other hand, the rate of tumor implantation in vivo of HSC-3 BRAK cells (Fig. 1 BRAK) was significantly lower than that of HSC-3 Mock ($P < 0.001$). In addition, the tumors formed in vivo by the HSC-3 BRAK cells were significantly smaller than those of the mock vector-transfected ones. By 27 days after xenografting, there was no tumor observed when HSC-3 BRAK cells were injected (Fig. 2, C and D). In these experiments, tumors less than 5 mm$^3$ in volume were regarded as nonimplanted (suppressed). In fact, only fat tissues and/or scar tissues were observed in the injected sites; and no tumor cells were detected by histological examination of the remnant tissues. These results indicate that BRAK/CXCL14 is a chemokine, having a tumor suppressive activity toward tumor progression of OC in SCID mice.

All nude mice are T cell deficient and accept tumors derived from some other species. We inoculated HSC-3 BRAK or HSC-3 Mock cells subcutaneously into both sides of the back region of 10 female nude mice (BALB/cAJcl-nu-nu; Clea Japan, Tokyo) and compared implantability of xenografts with those of SCID mice. Implantability of HSC-3 Mock cells was 100% at day 3 after tumor cell injection both SCID and nude mice; but after 27 days, only 80% of tumor cells retained in nude mice, being lower than the 100% for the SCID mice (Table 1). In contrast, in the case of BRAK-expressing tumor cells in the SCID mice, only 35% of xenografts retained at day 3 after tumor cell injection both SCID and nude mice; but after 27 days, only 80% of tumor cells retained in nude mice, being lower than the 100% for the SCID mice (Table 1). In addition, no BRAK-expressing tumor cells were detected in SCID mice.

![Fig. 1](image1.png)

**Fig. 1** Effect of BRAK expression on HSC-3 tumor xenografts in SCID mice. Pooled clones of BRAK-expressing (BRAK) and mock vector-transfected (Mock) cells ($5 \times 10^6 / \text{site}$) were inoculated subcutaneously into both sides of the back region of 10 female SCID mice. Mock: open circles, average of 20 tumors in 10 animals. BRAK: closed circles, average of 20 tumors in 10 animals. A significant difference in the size of tumors was observed at all points measured. $P < 0.001$ (Student's t-test).

![Fig. 2](image2.png)

**Fig. 2** Photographs of tumor cell xenografts at 27 days after xenografting. A mixture of BRAK/CXCL14-expressing cell clones or mock vector-transfected cells was inoculated subcutaneously into both sides of the back region of 10 female SCID mice. A and B, mice injected with mock vector-transfected cells; C and D, mice injected with BRAK-expressing cells. Arrowheads point out the tumors (A, B) or injection site (C, D).
The suppressor effect of the drug was not observed in the case of BRAK non-expressing cells. Furthermore, introduction of small interference RNA expression-vector for BRAK into HSC-3 cells reduced both the expression levels of BRAK in HSC-3 cells and the antitumor efficacy of gefitinib in vivo. These data also indicate that BRAK expression is essential for tumor suppression in vivo (14). The same or a higher level of natural killer (NK) cell activity is present in SCID mice compared with wild-type C.B-17 mice (26); and BRAK/CXCL14 stimulates the migration of activated NK cells but does not affect the proliferation or cytotoxic activity of normal NK cells (21), suggesting that NK cells are partially responsible for BRAK/CXCL14-dependent tumor suppression and that the co-presence of a NK cell activator(s) is essential for tumor suppression. It is also reported that tumor angiogenesis is important for tumor growth and progression (4, 12, 23) and that BRAK/CXCL14 was shown to have angiostatic (anti-angiogenic) activity (17). Thus, inhibition of tumor angiogenesis might be also important for the suppression of tumor progression.

In conclusion, this study indicates that expression of BRAK/CXCL14 in tumor cells suppresses the very early stage of tumor establishment in SCID mouse xenografts, suggesting the possibility that BRAK/CXCL14 expression inhibits tumor stem cell establishment by enhancing interactions between tumor stem cells and NK cells and/or by suppressing the formation of tumor microvessels. Our finding may be important for clarification of the molecular mechanisms of tumor suppression by this endogenous chemokine, BRAK/CXCL14.

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Table 1  Comparison of implantability of tumor cell xenografts between SCID and nude mice

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The same mixture of BRAK-expressing (BRAK) or mock vector-transfected (Mock) cells (5 × 10⁶/site) was subcutaneously inoculated into both sides of back region of 10 female SCID or Nude mice. Size of tumors was measured every 3 or 4 days. Tumors were regarded as nonimplanted when the tumor size was less than 5 mm³.
REFERENCES