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Letter to the Editor

Sympathetic Nervous System Derived Trophic Factor Augments Growth of Human Neuroblastoma *In Vitro*

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NEUROBLASTOMA is one of the most common solid tumors of infancy and early childhood. Neuroblastoma and the sympathetic nervous system (SNS) share a common embryological origin, the neural crest, and as a result both share many neural properties. We have shown previously that SNS modulates growth of mouse neuroblastoma (C1300 NB) *in vivo* and *in vitro*. We have shown that ablation of the SNS in mice by chemical treatment prior to C-1300 NB injection suppressed growth of this tumor significantly [1]. On the contrary, pretreatment of mice with NGF, which produces overgrowth of the SNS, prior to tumor injection augmented growth of NB [2]. We have also reported that coculturing of C-1300 NB explants with superior cervical ganglia (SCG) obtained from newborn rats augments growth of NB [3]. Furthermore, we showed that media conditioned by SCG as well as supernatants from freshly excised SCG augment growth of C-1300 NB, and its clonal line S-20, but do not influence growth of C-1300 NB clonal lines N1E 115 and C-46 *in vitro*. Growth of C-1300 NB and S-20 *in vivo* was suppressed significantly in mice with destroyed SNS, but growth of N1E 115 and C-46 was not affected by ablation of SNS [4]. Our experiments suggest that the SNS secretes a trophic/mitogenic factor which favors growth of C-1300 NB and some of its clonal lines. We now present data on the influence of the SCG trophic/mitogenic factor on adrenergic and cholinergic lines of human neuroblastoma.

Superior cervical ganglia (SCG) were obtained from 1 day old Wistar–Furth rats. Four SCG were suspended in 1 ml of minimum essential medium (MEM) with Earle's salts (Gibco Labs, Grand Island, NY), sonicated on ice for 3–5 s using a Branson Sonifier Cell Disrupter 18 S, and then filtered through a 0.22 μ microfilter. The protein content of SCG filtrate obtained from 4 SCG averages 22 μg/ml.

Human neuroblastoma cell lines SK-N-MC (cholinergic line) and SK-N-SH (adrenergic line) were obtained from American Type Culture Collection. Cells were carried in culture in MEM with Earle's salts containing 10% FBS, 2% L-glutamine and 1% gentamicin. For experiments, SK-N-SH or SK-N-MC cells were seeded onto (9 cm^2) Petri dishes at 5×10^4 cells/dish. Cells in experimental dishes were cultured in MEM with Earle's salts with 1% FBS and 0.5 ml of SCG filtrate/dish. Cells cultured in MEM with Earle's salts with 1% FBS served as a control. Cells were incubated at 37°C in humidified 5% CO_2 balanced with air for 72 h. For each experiment duplicate dishes were studied. After 72 h in culture, cells were removed and viable cells were counted. Statistical analyses were done using Student's *t*-test.

Growth of SK-N-SH human neuroblastoma cell line was significantly augmented in the presence of newborn rat SCG filtrate. The number of SK-N-SH cells grown in the presence of SCG filtrate was $3.3 \pm 0.32 \times 10^5$ cells/dish. The number of cells grown in control medium was 1.8 ±

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0.13×10^5 cells/dish ($n = 4$). The difference was statistically significant at $P < 0.01$.The growth of SK-N-MC cells was not affected by the presence of SCG filtrate. The number of cells grown in the culture medium containing SCG filtrate was $1.8 \pm 0.15 \times 10^5$ cells/dish and in control medium was $1.7 \pm 0.20 \times 10^5$ cells/dish ($n = 4$). The difference was not significant.

We have shown that a trophic/mitogenic factor present in newborn rat SCG augments the growth of adrenergic cell line of human neuroblastoma (SK-N-SH), but does not influence growth of cholinergic line SK-N-MC *in vitro*. In earlier work we have shown that SNS modulates growth of mouse neuroblastoma *in vivo* and *in vitro*. Growth of C-1300 NB and its clonal line S-20 was significantly suppressed in mice with chemically destroyed SNS and significantly augmented *in vitro* in the presence of SCG explants, SCG conditioned medium, or newborn SCG filtrate. We have also shown that a trophic/mitogenic factor, which is present in newborn SCG, is absent in adult animals [5]. It is tempting to link the presence of this factor in SCG obtained from young animals to the occurrence of NB in young individuals. We have also shown that a trophic/mitogenic factor present in newborn SCG is relatively specific for neuroblastoma since it did not influence growth of mouse A-10 breast adenocarcinoma or C6 glioma *in vitro*. Neurotrophic effects on growth and differentiation are well documented, however, little is known about neurotrophic effects on tumor growth [6–11]. To our knowledge only one other group of investigators, in addition to ourselves, studied growth of neuroblastoma in denervated tissue. They showed that growth of NB tumor in denervated muscle was retarded and NB cells grown in denervated tissue had significantly lower mitogenic rate than control tumors [12, 13]. We do not know at present why sensitivity to SNS factor varies between different clonal lines of neuroblastoma. It may relate to specific neural properties of neuroblastoma cells or to the presence of receptors for the SNS trophic factor in selected neuroblastoma cells.

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