Effects of Fibroblast Growth Factors 19 and 20 on Cell Multiplication and Locomotion in a Human Embryonal Carcinoma Cell Line (Tera-2) In Vitro

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Abstract. Background: The effects of two recently discovered heparin binding growth factors, FGF-19 and FGF-20, on the human embryonal carcinoma derived cell line Tera 2 were examined. Materials and Methods: Cell numbers, as well as cell migration were examined at the clonal level by light microscopy. Results: FGF-19, as well as FGF-20 promoted Tera 2 cell multiplication. Whereas FGF-20 promoted cell multiplication at low doses, FGF-19 was required at high doses to achieve a comparable effect. Moreover, FGF-19 did not significantly stimulate cell locomotion, while FGF-20 promoted cell motility at high doses. Conclusion: FGF-19 and FGF-20 qualitatively exert different effects on cell survival and cell locomotion.

The fibroblast growth factors (FGF) play an important, but not yet fully understood, role in the regulation of important cellular processes. The FGF family consists of 23 members ranging in size from 17 to 34 kDa and the last member was discovered more than five years ago (1). These polypeptides share a common core of approximately 140 amino acids (2). Whereas the first two members of the FGF family, acidic and basic FGF lack a signal peptide required for secretion, most other members of the FGF family have retained this leader sequence (2).

FGFs bind chiefly to two types of structures. FGF molecules form a low affinity interaction with heparan sulphate, an important structural component of the extracellular matrix. FGFs also bind to FGF-receptors (FGF-R 1-5 in mammals). Many of the diverse effects of the FGFs have been explained by their different affinities to different receptors (3).

This study was aimed at examining the effects of two recently characterised members of the heparin binding growth factor family (FGF-19 (4) and FGF-20 (5)), that have both been assigned important regulatory roles in development. A well characterised human embryonal carcinoma cell line, Tera 2, frequently used for simultaneous studies of proliferation and locomotion on a clonal level (6-9), was employed. It is demonstrated here that while FGF-19 exerts an effect on proliferation at low concentrations but no effect on motility, FGF-20 exerts effects on proliferation at low concentrations and on motility at higher concentrations.

Materials and Methods

Cell culture. Human embryonal carcinoma-derived Tera 2 cells (Clone 13) were routinely passaged as described by Thompson et al. (10) in alpha medium supplemented with 10% heat-inactivated fetal calf serum (FCS) (GIBCO). Serum-free medium was essentially as described by Biddle et al. (11). Briefly, it consisted of a 1:1 (v/v) mixture of alpha medium (lacking nucleosides and deoxyribonucleosides) and Hams F12 medium (GIBCO) supplemented with 10 ìg/ml of human transferrin preloaded with iron in accordance with the manufacturer's instructions.

The cells used for the growth experiments were plated out at a density of 1.5-3.5x10^5 cells in each 6-cm diameter Primaria dish (Becton-Dickinson) and were allowed to attach overnight in 10% FCS. The following day, the cells were counted and this was taken as the starting point of the experiment. The counting of cells exposed to different experimental media was carried out after exposure of washed monolayers to 0.125% (w/v) trypsin, 0.0125% (w/v) EDTA for 10 min at room temperature to ensure complete harvesting. The range of cell counts in the duplicate and triplicate dishes only rarely exceeded 8% of the total cell count. The results of these counts were normalised by relating the number of population doublings since the start of the experiment, as described in detail in Biddle et al. (11).
Growth factors. Recombinant FGF-19 and FGF-20 were purchased from Research Diagnostics Inc, (Concord, Mass, USA). Protamine sulphate was purchased from Sigma, Sweden.

Clonal analysis. Tera 2 cells were plated at different densities ranging from 10 to 100 cells per square centimetre in equilibrated medium in 35 mm wells of six cluster dishes. After a five-day exposure to the experimental media, each culture was fixed in 3:1 (v/v) methanol: acetic acid for at least 24 h, hydrated in Analar water and finally stained with 1% (w/v) aqueous crystal violet. Each dish was examined under a Leitz inverted microscope equipped with an eyepiece graticule inscribed with 24 concentric circles. The distance between two neighbouring circles was taken as one relative unit. Image analysis using a C-scan (Cambridge) gave identical results to the manual method. Each dish was subsequently scanned in the microscope and the location of each visible colony marked. The diameter of each colony was determined by the use of the graticule, whereafter the number of cells in each colony was counted. In several instances, the colonies were photographed under a Leitz inverted microscope with an attached camera system. This procedure was described in detail elsewhere (7).

Results

Growth effects. Daily additions of FGF-19 or FGF-20 to Tera 2 cells plated out in serum-free medium over a five-day period showed that multiplication of these cells could be supported by 10 µg per ml of any one of these growth factors, as the sole macromolecular additive. This resulted in at least one-half population doubling over the time course of the experiment and compared with controls that showed essentially no increase in cell number (Figure 1).

However, the magnitude and dose response differed between the two factors. Whereas FGF-20 reached its dose optimum in the interval 10-100 µg/ml and then tailed off, FGF-19 increased its multiplication stimulating activity with increasing growth factor concentration and reached its maximum at 100 µg/ml; thereafter, the effect tailed off. In neither case could any further significant effect be achieved by adding a higher concentration of the growth factor.

The effects of FGF-19 and FGF-20 were both abrogated, if 10 µg protamine sulphate were concomitantly added in a manner previously described for bFGF (6) (data not shown) and inclusion of this agent in control experiments was hence used as a control of the specific effect of each of the two heparin binding growth factors.

Cell locomotion. To study the relationship between cell multiplication and migration, Tera 2 cells were seeded at clonal density and grown with different concentrations of FGF-19 or FGF-20. In this set of experiments, the cell number, as well as the relative diameter was scored for each colony. In order to exclude the possibility that any of the observed effects on cell migration were a consequence of an enhanced cell multiplication, the diameter of colonies that contained comparable cell numbers were examined in the presence or absence of the two growth factors under study.

It was found that FGF-19, as well as FGF-20 stimulated clonal proliferation as determined by the number of cells per colony. Moreover, as shown in Table I, irrespective of colony size as defined by number of cells per colony, the effects on cell motility (as compared to the controls) were best achieved when high doses (50-100 µg/ml) were added. There was a conspicuous difference between the two factors in that FGF-19 did not result in any statistically significant effect on cell locomotion. In contrast, FGF-20 significantly stimulated cell locomotion only when added at high concentrations (100 µg/ml).

In the case of FGF-19, there was no significant difference in colony size as compared to the controls in the range 5-100 µg/ml. However, for FGF-20 this effect was only significant for the highest concentration tested (100 µg/ml). The stimulatory effects on cell migration were ascribable to the addition of FGF-20 itself, since its effects were abrogated by simultaneous addition of protamine sulphate (data not shown).

Discussion

In their classic study, McAvoy and Chamberlain showed that the two archetypical fibroblast growth factors, aFGF and bFGF, were capable of inducing different biological responses when supplied at different doses. When the growth factors were added to chick lens epithelial cells, preferential effects
were observed on proliferation, locomotion of differentiation (as determined by activation of the beta-crystalline gene) depending on the growth factor concentration (12-14). This plethora of effects executed by a single growth factor fits rather well into the pattern of multiple effects of FGFs observed during normal and tumour angiogenesis. We previously observed that the addition of nine different members of the FGF family gave rise to an increase in human teratocarcinoma cell numbers at very modest concentrations (6, 8, 9, 15). When the growth factor concentration was increased, a preferential effect on cell migration was observed. It was firmly concluded that the effect on cell numbers was chiefly due to an enhanced cell survival rather than recruitment of more cells into the cell cycle or an increased rate of cell cycle traverse (13).

In this study, we examined the effects of the two recently discovered growth factors, FGF-19 and FGF-20, on multiplication and motility at the clonal level. It was found that FGF-20 behaved in a fashion similar to all other examined members of the FGF family. FGF-20 exerted its maximum effect on clonal multiplication at low concentrations, whereas its effect on cell locomotion became most obvious at higher concentrations. In contrast, FGF-19 did not support Tera 2 cell multiplication at low concentrations, but a significant effect was achieved at high concentrations. Like other FGFs, FGF-20 stimulated cell motility at high concentrations, but this effect was conspicuously absent when FGF-19 was added.

It is reasonable to assume that the difference between FGF-19 and all other examined members of the FGF-family may relate to its receptor binding properties. FGFs bind to a class of FGF-receptors, where new isoforms with different ligand-binding properties are generated by alternative splicing mechanisms. There is an interesting difference between FGF-R1-3 and FGF-R4 in that the latter seems to lack alternative splice sites (9). It was recently shown that FGF-19 is unique in the sense that it only binds to one sole receptor - the FGF-R4. This exclusivity was explained by the unique structural properties of FGF-19 and FGF-R4. (16). This unusual affinity may well explain the absence of FGF-19 effects on cell motility since this effect is generally believed to be exerted via FGF-R2 (17). Since FGF-R2 is expressed in Tera 2 cells, it was assumed that this receptor is mainly responsible for its motility. However, older data suggest that membrane ruffling activities in malignant cells can be mediated via the FGF-R4 (18). Thus, the exact biological effects mediated by each receptor need to be further elucidated.

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References


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