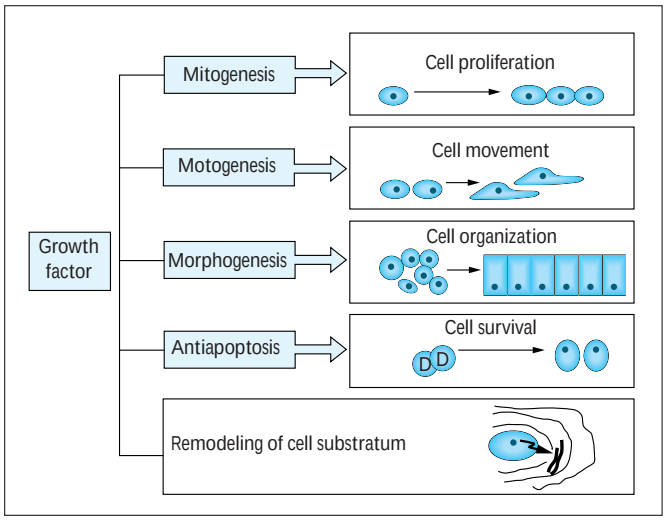

FIGURE 16-16

Cellular response to growth factors. Schematic representation of the pleiotrophic effects of growth factors, which share several properties and are believed to be important in the development and morphogenesis of organs and tissues, such as those of the kidney. Among these properties are the ability to regulate or activate numerous cellular signaling responses, including proliferation (mitogenesis), motility (motogenesis), and differentiation (morphogenesis). These characteristics allow growth factors to play critical roles in a number of complex biological functions, including embryogenesis, angiogenesis, tissue regeneration, and malignant transformation [83].

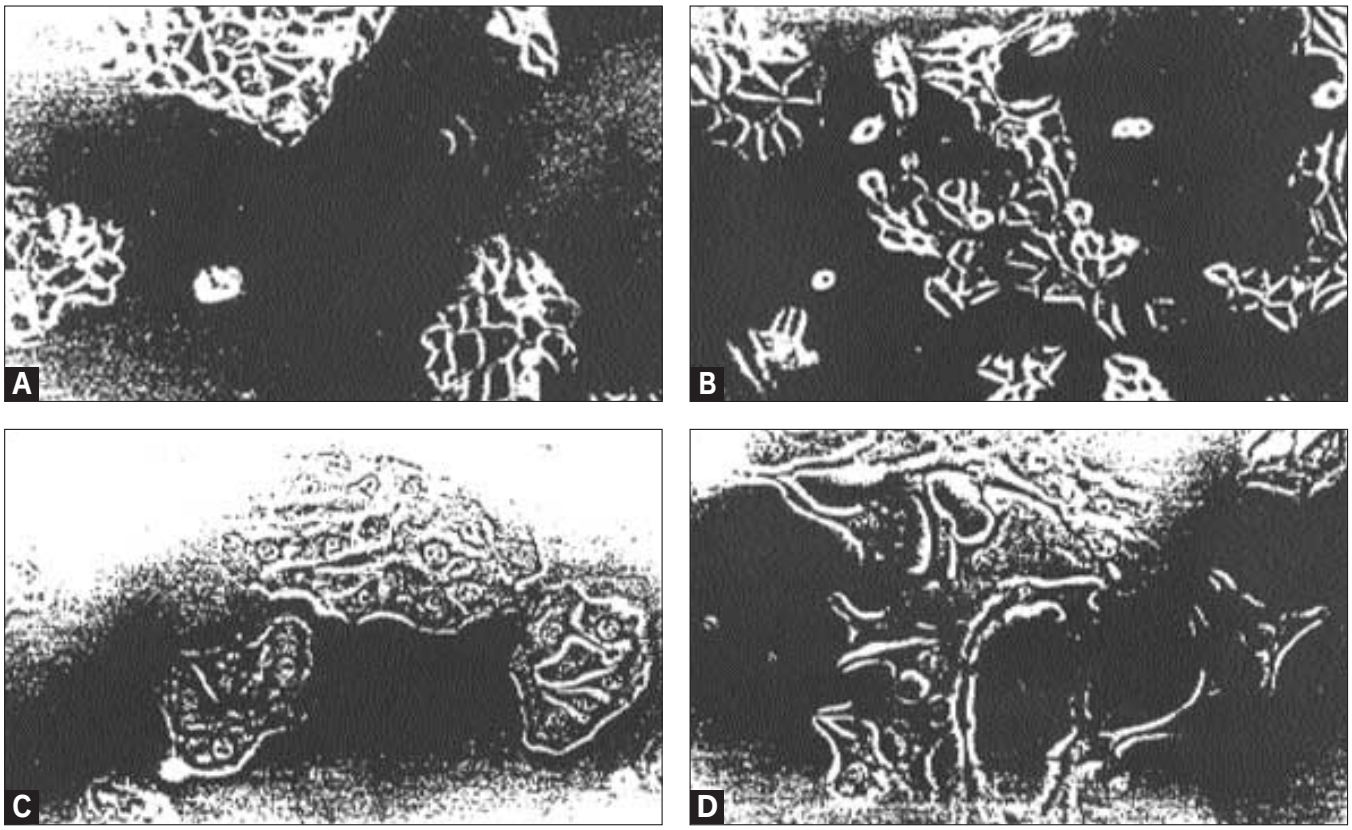

FIGURE 16-17

Motogenic effect of growth factors—hepatocyte growth factor (HGF) induces cell "scattering." During development or regeneration the recruitment of cells to areas of new growth is vital. Growth factors have the ability to induce cell movement. Here, subconfluent monolayers of either Madin-Darby canine kidney (MDCK) **C, D**, or murine inner medullary collecting duct (mIMCD) **A, B**, cells were grown for 24 hours in the absence, **A, C**, or presence **B, D**, of 20 ng/mL HGF. Treatment of either type of cultured renal epithelial cell with HGF induced the dissociation of islands of cells into individual cells. This phenomenon is referred to as scattering. HGF was originally identified as *scatter factor*, based on its ability to induce the scattering of MDCK cells [83]. Now, it is known that HGF and its receptor, the transmembrane tyrosine kinase *c-met*, play important roles in development, regeneration, and carcinogenesis [83]. (*From* Cantley *et al.* [84]; with permission.)

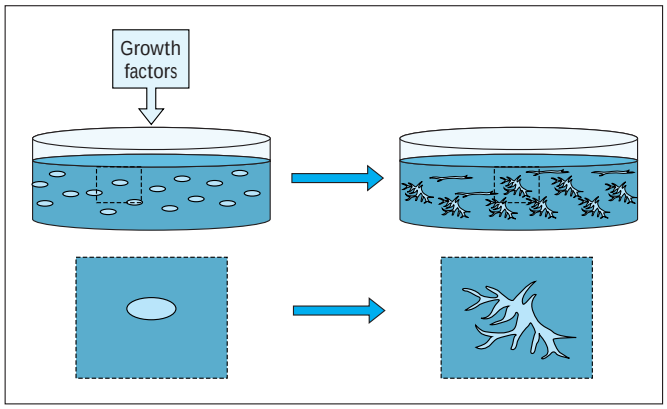

FIGURE 16-18

Three-dimensional extracellular matrix gel tubulogenesis model. Model of the three-dimensional gel culture system used to study the branching and tubulogenesis of renal epithelial cells. Analyzing the role of single factors (*ie*, extracellular matrix, growth factors, cell-signaling processes) involved in ureteric bud branching tubulogenesis in the context of the developing embryonic kidney is an extremely daunting task, but a number of model systems have been devised that allow for such investigation [77, 79, 85]. The simplest model exploits the ability of isolated kidney epithelial cells suspended in gels composed of extracellular matrix proteins to form branching tubular structures in response to growth factors. For example, Madin-Darby canine kidney (MDCK) cells suspended in gels of type I collagen undergo branching tubulogenesis reminiscent of ureteric bud branching morphogenesis in vivo [77, 79]. Although the results obtained from such studies in vitro might not correlate directly with events in vivo, this simple, straightforward system allows one to easily manipulate individual components (*eg*, growth factors, extracellular matrix components) involved in the generation of branching epithelial tubules and has provided crucial insights into the potential roles that these various factors play in epithelial cell branching morphogenesis [77, 79, 84–87].

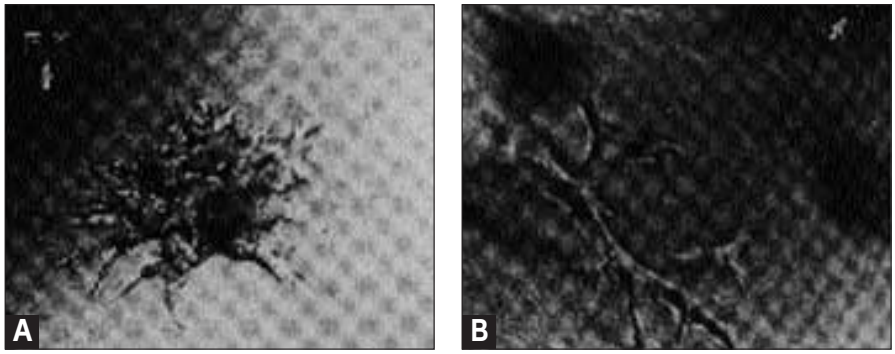

FIGURE 16-19

An example of the branching tubulogenesis of renal epithelial cells cultured in three-dimensional extracellular matrix gels. Microdissected mouse embryonic kidneys (11.5 to 12.5 days) were cocultured with **A**, murine inner medullary collecting duct (mIMCD) or, **B**, Madin-Darby canine kidney (MDCK) cells suspended in gels of rat-tail collagen (type I). Embryonic kidneys (EK) induced the formation of branching tubular structures in both mIMCD and MDCK cells after 48 hours of incubation at 37°C. EKs produce a number of growth factors, including hepatocyte growth factor, transforming growth factor-alpha, insulin-like growth factor, and transforming growth factor–β, which have been shown to effect tubulogenic activity [86–93]. Interestingly, many of these same growth factors have been shown to be effective in the recovery of renal function after acute ischemic insult [21–30]. (*From* Barros *et al.* [87]; with permission.)

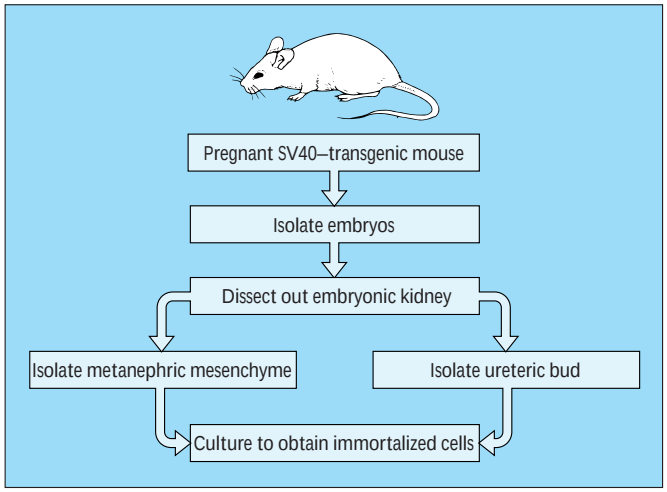

FIGURE 16-20

Development of cell lines derived from embryonic kidney. Flow chart of the establishment of ureteric bud and metanephric mesenchymal cell lines from day 11.5 mouse embryo. Although the results obtained from the analysis of kidney epithelial cells—Madin-Darby canine kidney (MDCK) or murine inner medullary collecting duct (mIMCD) seeded in three-dimensional extracellular matrix gels has been invaluable in furthering our understanding of the mechanisms of epithelial cell branching tubulogenesis, questions can be raised about the applicability to embryonic development of results using cells derived from terminally differentiated adult kidney epithelial cells [94]. Therefore, kidney epithelial cell lines have been established that appear to be derived from the ureteric bud and metanephric mesenchyme of the developing embryonic kidney of SV-40 transgenic mice [94, 95]. These mice have been used to establish a variety of "immortal" cell lines.

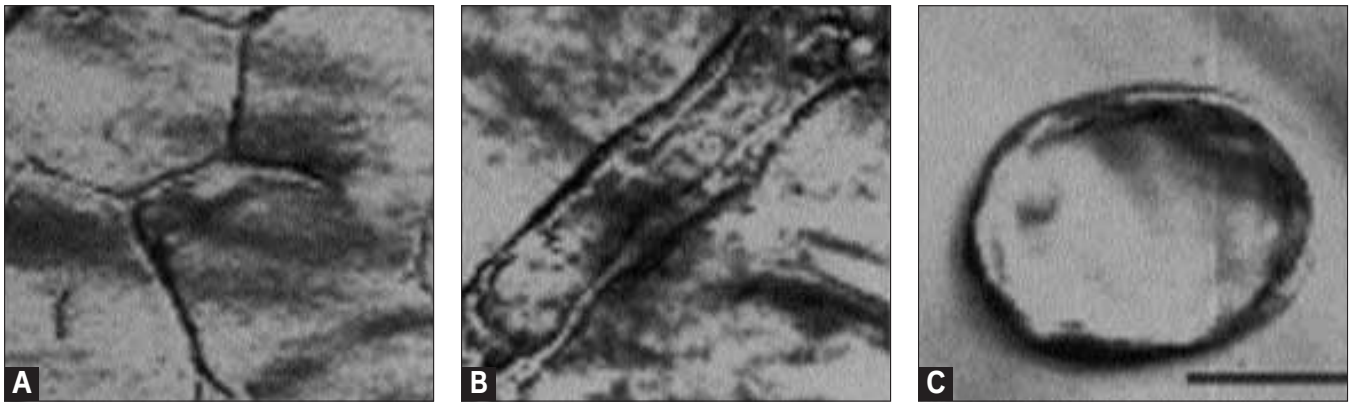

FIGURE 16-21

Ureteric bud cells undergo branching tubulogenesis in three-dimensional extracellular matrix gels. Cell line derived from ureteric bud (UB) and metanephric mesenchyme from day 11.5 mouse embryonic kidney undergo branching tubulogenesis in three-dimensional extracellular matrix gels. Here, UB cells have been induced to form branching tubular structures in response to "conditioned" media collected from the culture of metanephric mesenchymal cells. During normal kidney morphogenesis, these two embryonic cell types undergo a mutually inductive process that ultimately leads to the formation of functional nephrons [74–76]. This model system illustrates this process, ureteric bud cells being induced by factors secreted from metanephric mesenchymal cells. Thus, this system could represent the simplest in vitro model with the greatest relevance to early kidney development [94]. **A**, UB cells grown for 1 week in the presence of conditioned media collected from cells cultured from the metanephric mesenchyme. Note the formation of multicellular cords. **B**, After 2 weeks' growth under the same conditions, UB cells have formed more substantial tubules, now with clear lumens. **C**, Interestingly, after 2 weeks of culture in a three-dimensional gel composed entirely of growth factor–reduced Matrigel, ureteric bud cells have not formed cords or tubules, only multicellular cysts. Thus, changing the matrix composition can alter the morphology from tubules to cysts, indicating that this model might also be relevant to renal cystic disease, much of which is of developmental origin. (*From* Sakurai *et al.* [94]; with permission.)

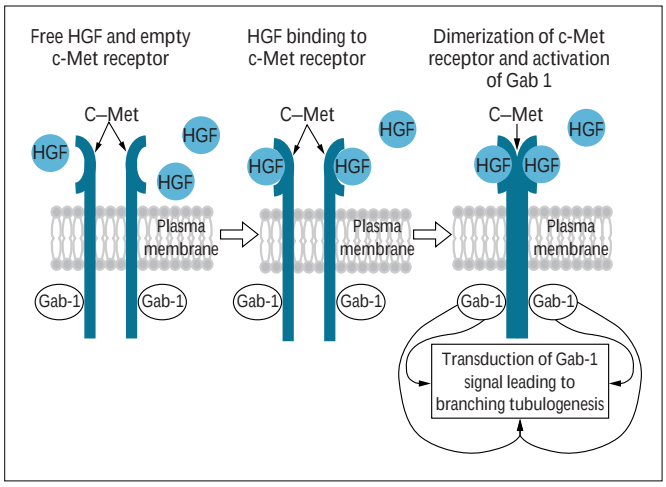

FIGURE 16-22

Signalling pathway of hepatocyte growth factor action. Diagram of the proposed intracellular signaling pathway involved in hepatocyte growth factor (HGF)–mediated tubulogenesis. Although HGF is perhaps the best-characterized of the growth factors involved in epithelial cell-branching tubulogenesis, very little of its mechanism of action is understood. However, recent evidence has shown that the HGF receptor (c-Met) is associated with Gab-1, a docking protein believed to be involved in signal transduction [96]. Thus, on binding to c-Met, HGF activates Gab-1–mediated signal transduction, which, by an unknown mechanism, affects changes in cell shape and cell movement or cell-cell–cell-matrix interactions. Ultimately, these alterations lead to epithelial cell–branching tubulogenesis.

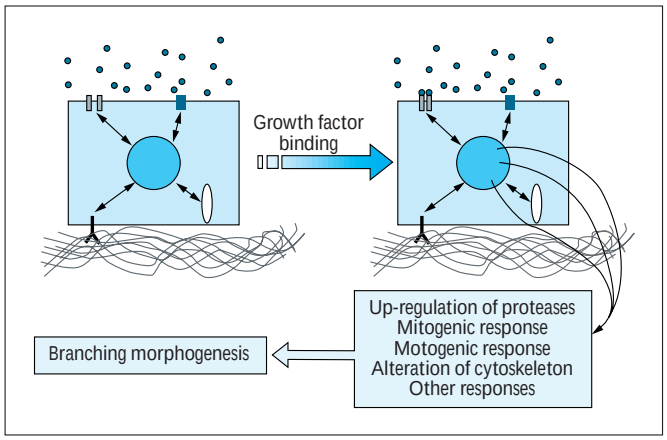

FIGURE 16-23

Mechanism of growth factor action. Proposed model for the generalized response of epithelial cells to growth factors, which the depends on their environment. Epithelial cells constantly monitor their surrounding environment via extracellular receptors (*ie*, integrin receptors) and respond accordingly to growth factor stimulation. If the cells are in the appropriate environment, growth factor binding induces cellular responses necessary for branching tubulogenesis. There are increases in the levels of extracellular proteinases and of structural and functional changes in the cytoarchitecture that enable the cells to form branching tubule structures.