not observed for the mature form, and that proHB-EGF may act as a tumor survival factor in hepatoma cells.

Hepatomas at the earliest stage do not always need rapid growth, but it is necessary for escape from various immune systems. Resistance against several factors may play a role in the early progression of hepatomas. A hepatoma overexpressing proHB-EGF showed strong resistance to several factors. Although the mechanism underlying resistance to TGF β -induced

apoptosis remain unknown, the resistance to serum-starved treatment is thought to be due to G1 arrest induced by up-regulation of p21. In some cases, cells under G1 arrest undergo apoptosis. However, pHB-AH cells showed slow growth with a high percentage of the G1 phase in the cell cycle. Thus, proHB-EGF specifically may act as a cell survival factor in liver tissues.

6. Apoptosis in Neurodegenerative Disorders

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Apoptosis has been implicated as a mechanism of neuronal degeneration in many neurodegenerative disorders. We report recent progress in apoptosis in Parkinson's disease (PD) and other major neurodegenerative disorders. We studied autopsied midbrains from 11 patients with Parkinson's disease and 6 control patients who died from various causes but not from neurodegenerative disorders; the details have been reported elsewhere (1). Four out of the 11 PD patients were of young onset PD (onset before 40 years of the age) and the remaining 7 were late onset sporadic patients. We used the standard TUNEL method to detect apoptosis. None of the control and young onset PD patients showed TUNEL positive neurons in the substantia nigra. Four of the 7 late onset PD patients had TUNEL-positive neurons in the substantia nigra. Counting the TUNEL-positive neurons in one of the midbrain sections revealed that 0.6 to 4.8% (mean 2.1%) of the remaining nigral neurons were TUNEL positive in those 4 patients. Although the percentages were low, we believe that apoptosis is involved in nigral cell death in PD. The low percentage is understandable considering that fragmented nuclei are quickly phagocytosed by glia cells, and the chance is small that fragmented DNA can be picked up by the TUNEL method. TUNEL-positive astrocytic and oligodendrocytic nuclei were also seen in some patients; 6 out of 7 late onset PD patients, 2 out of 4 young onset PD patients, and 1 out of 6 control patients had TUNEL positive glia cells. The reason why glia cells were more TUNEL positive may be due to the fact that glia cells have to be removed from the tissue when their roles are over.

According to the literature, Dragunow et al (2) studied 3

patients with PD, but they could not detect TUNEL-positive neurons. Anglade et al (3) studied 3 PD patients, and Tompkins and Hill (4) studied 28 patients with Lewy body diseases including PD, and both groups found evidence of apoptosis in all of the patients studied. It has been claimed that TUNEL does not discriminate apoptosis from necrosis (5), but another report stated that TUNEL stained more apoptosis than necrosis, although TUNEL is not 100% specific for apoptosis (6).

Although histochemical evidence of apoptosis in PD is not sufficient, there is biochemical evidence which supports the presence of apoptosis in PD. It has been well known that in PD, mitochondrial complex I and α -ketoglutarate dehydrogenase complex are decreased in the substantia nigra (7–9); therefore, mitochondrial respiration is impaired in PD, which causes an increase in the formation of oxygen free radical species, because of incomplete reduction of oxygen to water. There is ample evidence to indicate oxidative stress in PD (10, 11).

According to the recent progress in the study of the mechanism of apoptosis, loss of mitochondrial inner membrane potential ($\Delta\Psi_m$) has been found to be one of the major triggers of apoptosis (12). This membrane potential is achieved by electron transport in which protons are expelled from the matrix space to the intermembrane space; thus the matrix side becomes negatively charged. Induction of apoptosis by mitochondrial respiratory toxins have been described (13, 14). Recently, Susin et al (15) have shown that a 50-kDa protein is released from the mitochondrial intermembrane space to cytoplasm, and this protein induces nuclear fragmentation and apoptosis when applied to the nucleus. Further, opening of permeability transi-

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tion pores which are present in the inner membrane of mitochondria has been recently postulated as the initial mediator of loss of mitochondrial membrane potential (16, 17) which ultimately induces apoptosis. Opening of the permeability transition pores increases the permeability of the inner mitochondrial membrane and thus dissipates the membrane potential. Interestingly, oxygen free radicals are able to open these permeability transition pores (18). When we review the recent progress in the study of the role of mitochondria in the induction of apoptosis, early observation of loss of complex I in PD is a very important step to elucidate the pathogenesis of PD. We believe that there is ample evidence to believe that apoptosis plays a very important role in the nigral cell death in PD.

In Alzheimer's disease, Su et al (19) and Lassmann et al (20) observed many TUNEL-positive cortical neurons. Recently, Wolozin et al (21) reported that presenilin 2 has a facilitatory action on apoptosis in cultured cells; mutation of presenilin 2-gene is responsible for one type of autosomal dominant familial Alzheimer's disease. In Huntington's disease, TUNEL-positive neurons have been found in the striatum (22). Further, abnormally elongated polyglutamine stretch appears to enhance apoptosis (23).

Thus the accumulating evidence indicates the involvement of apoptosis in various neurodegenerative disorders. Under the supposition that apoptosis is an important mechanism of neuronal death, it may become possible to stop or retard the degenerative process by interrupting apoptosis in the future. Further studies on apoptosis in neurodegenerative disorders are very important from the therapeutic aspect.

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