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Review

Cytomegalovirus infection blocks apoptosis in cancer cells

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Abstract. Recent pathological findings reveal a higher frequency of human cytomegalovirus (HCMV) in tumor cells from different tumors compared with surrounding tissues. Experimental investigations suggest possible supportive effects of HCMV for tumor development and progression. One HCMV effect on tumor cells is the inhibition of apoptosis, leading to the promotion of tumor cell survival. Decreased sensitivity to treatment-induced tumor cell death is a major reason for failure of anticancer chemotherapy. HCMV infection interferes with both the intrinsic and extrinsic cellular apoptosis pathways. HCMV promotes cell survival signaling influencing the tumor suppressor p53 and its relative p73, and stimulates the antiapoptotic Ras/Raf/MEK/Erk- and PI-3K-signaling pathways. Antiapoptotic effects mediated by HCMV are inhibited by antiviral treatment in cell culture. Therefore, a better understanding of the influence of HCMV infection on tumor cell apoptosis might translate into improved anti-cancer therapy.

Key words. Cytomegalovirus; apoptosis; tumor cells.

Introduction

Recent pathological investigations revealed that human cytomegalovirus (HCMV) can be found in colorectal cancer, glioma and prostate cancer cells with high frequency [1–3]. Moreover, detection of HCMV proteins which are expressed in infected cells only during viral replication demonstrated that persistent productive infection is necessary to sustain HCMV infection in tumor tissues [1–3]. These findings confirmed former observations that suggested HCMV plays a role in tumor development [4–14]. However, since inoculation of permissive normal human cells does not result in transformation, HCMV is not regarded as a 'classical' tumor virus but one that modulates the malignant properties of cancer cells. Oncomodulation by HCMV stems from its ability to interfere with a variety of cellular signal transduction pathways, leading to accelerated cell proliferation, enhanced survival, angiogenesis, cell motility and adhesion, thus enhancing the malignant behavior of tumor cells [15]. HCMV-infected tumor cells (possessing disturbed functioning signaling pathways, transcription factors and tumor suppressor proteins) provide the genetic background necessary for the oncomodulatory effects of HCMV, which cannot otherwise be manifested in normal cells.

To study the long-term effects of HCMV infection on the malignant properties of tumor cells, we established a preclinical model of persistent HCMV infection in cell lines derived from different tumors. Persistently infected tumor cell lines were able to grow and to produce infectious HCMV for extended periods of time [15–17]. Persistent productive infection was sustained by a balance between rapidly dividing noninfected tumor cells and infected tumor cells which promoted a complete replication cycle of HCMV. In this regard HCMV infection of tumor cells differs from persistent infection with DNA tumor viruses (e. g. human papilloma virus) that fail to proceed past the expression of early gene products. The significance of

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persistent productive infection for HCMV oncomodulatory activity is supported by findings demonstrating that enhancement of malignant properties may revert after HCMV elimination from tumor cells by treatment with antiviral drugs [16]. Although the mechanisms underlying the long-term persistence of HCMV in tumor cell cultures has not been determined, inhibition of apoptosis plays a pivotal role [16].

This review focuses on the apoptosis-inhibiting effects of HCMV. Deregulation of cell death programs plays a major role in the development of cancer. Cell proliferation and apoptosis ensure homeostasis of all tissues. Resistance to apoptotic stimuli is responsible for uncontrolled tumor cell survival and, consequently, expansion of malignant cells. This leads to genetic instability and accumulation of mutations. Moreover, sensitivity to anticancer treatment such as chemotherapy or radiation as well as to immune-mediated cell destruction is reduced [18]. In addition, decreased sensitivity to apoptosis plays an important role in metastasis formation due to the enhanced robustness of cancer cells [19]. Resistance to anticancer therapy and/or metastasis formation are major reasons for poor outcome in cancer patients. Therefore, the influence of HCMV on tumor cell apoptosis is a major aspect of the oncomodulatory potential of HCMV.

HCMV infection and death receptor signaling

The extrinsic apoptosis pathway is initiated by binding of specific ligands, such as FasL, TNF-α (tumor necrosis factor-α), Trail (TNF-related apoptosis-inducing ligand), and Apo-3LTweak to death receptors, such as Fas/Apo1/CD95, TNF receptor-1 (TNFR1), death receptor 3 (DR3), DR4, DR5 and DR6, resulting in receptor clustering and formation of a death-inducing signaling complex. TNFR1 binds TNF-α, Fas binds FasL, DR3 binds Apo-3L/Tweak, DR4 and DR5 bind Trail, whereas no ligand for DR6 has yet been identified [20]. Via the Fas-associated death domain protein (FADD) and TNF-related protein associated with death domain (TRADD), these complexes recruit multiple procaspase-8 molecules, resulting in caspase-8 activation [20–22].

HCMV modulates the extrinsic apoptosis pathway either by encoding viral proteins that directly interfere with the extrinsic apoptosis pathway or by altering expression of cellular proteins involved in death receptor signaling (fig. 1).

HCMV vICA (viral inhibitor of caspase activation), which is encoded by the viral UL36 gene, protects cells from apoptosis triggered by several death receptors, including TNFR1, Fas/CD95 or Trail [23]. Interestingly, UL36 is mutated in many HCMV laboratory strains, suggesting that vICA/pUL36 does not play an essential role in virus replication in vitro. vICA directly interacts with the caspase pro-domain, inhibiting the processing of procaspase-8 (FLICE) [23]. Therefore, the action of vICA appears to be similar to that of viral as well as cellular procaspase-8/FLICE-ligand inhibitory proteins (FLIPs), although there are no sequential or structural similarities between vICA and FLIPs. Consequently, vICA is able to modulate cytokine- and/or death receptor-mediated apoptosis.

Another mechanism by which HCMV might interfere with death receptor signaling is encoding of a TNFR ortholog via the HCMV UL144 orf [24]. Binding of TNF by soluble viral decoy receptors was recognized as a tumor virus immune escape mechanism, as shown by the secreted TNFR ortholog expressed by tumorigenic Shope fibroma virus (rabbit poxvirus) [25]. However, the functional significance of the HCMV TNFR ortholog remains to be proven [26].

HCMV immediate early antigen 1 (IE1) and 2 (IE2) prevent apoptosis by affecting death receptor signaling pathways, such as the TNF-mediated death receptor-signaling pathway [27]. However, it remains undetermined whether this apoptosis inhibition is provoked by direct interference with the extrinsic apoptosis pathway or mediation of apoptosis inhibition by cellular proteins, or whether the mechanism is a combination of both.

Moreover, two recent publications revealed that HCMV as well as murine CMV downregulate TNFR in different cell types, including the glioblastoma cell line U373 [28, 29]. HCMV disrupted the function of TNFR1 by relocalization and, therefore, by elimination of TNF-α-induced Jun kinase activity [28].

An interesting mechanism by which HCMV modulates extrinsic apoptotic pathways is through its stimulatory effect on expression of death receptor ligands, although stimulation of death receptor ligand expression can hardly considered to be an anti-apoptotic mechanism. It has been shown that HCMV IE2-86 upregulates membrane-bound FasL in human retinal pigment epithelial cells [30, 31] and induces Trail expression in fibroblasts [32]. This upregulation of death receptor ligands might cause apoptosis of infiltrating Fas- and DR4/DR5-positive immune cells, while HCMV-infected cells are protected from apoptosis through numerous mechanisms. Therefore, death receptor/ligand upregulation in connection with anti-apoptotic mechanisms is likely to be part of viral immune escape strategies [26, 30]. To date, the influence of HCMV infection on expression of death receptor ligands in tumor cells has not been investigated. Upregulation of death receptor ligands in connection with apoptosis inhibition in HCMV-infected cells might substantially contribute to the oncomodulatory properties of HCMV, especially considering that FasL expression by different tumor cells might be associated with immune escape, enhanced tumor progression, enhanced metastasis formation and local tissue destruction [33–36].

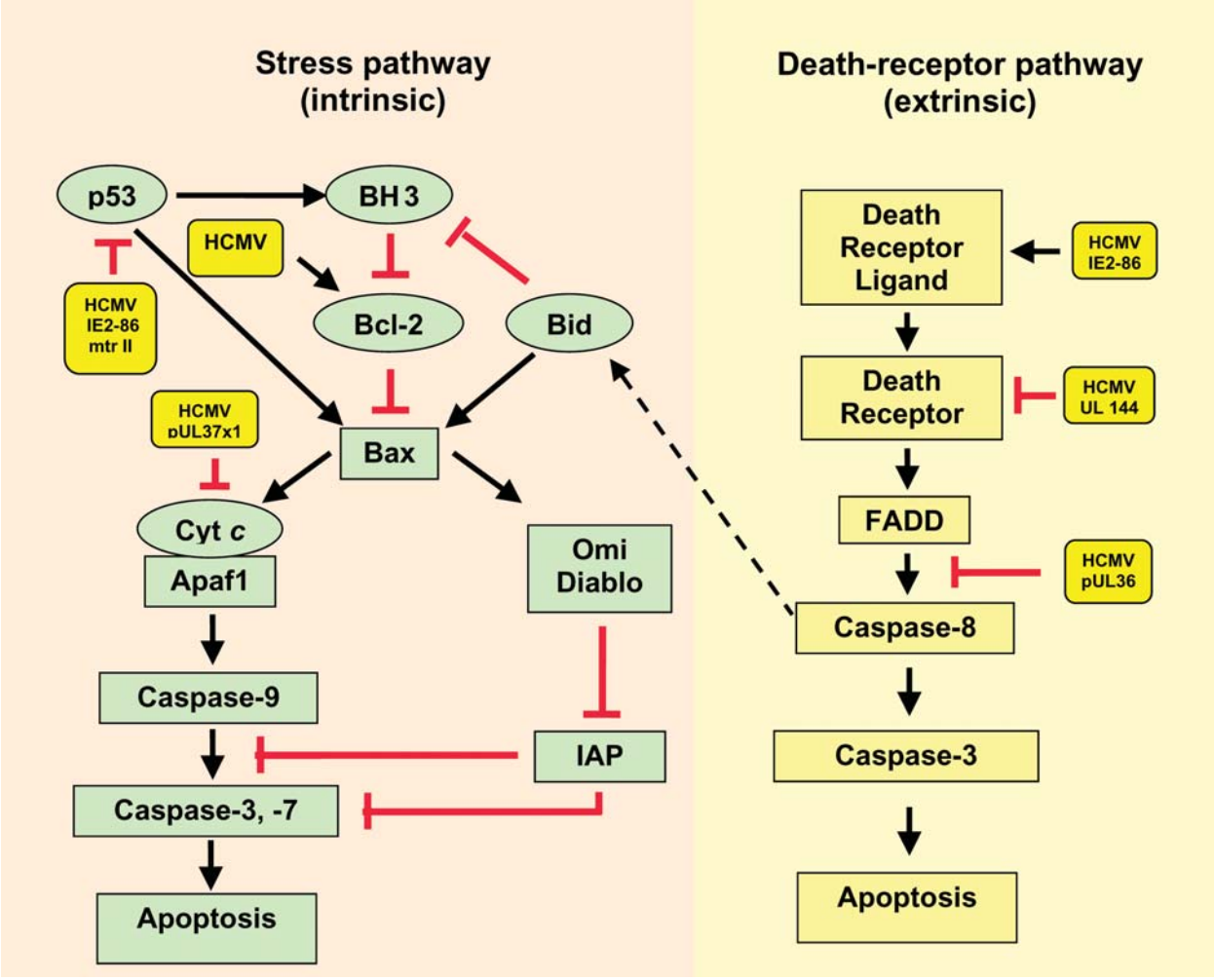

Figure 1. Interaction of HCMV regulatory proteins with the both two major apoptosis pathways both results in caspase activation. The intrinsic pathway mediates intracellular stress signals by mitochondrial release of Cyt *c*, which forms the apopotosome with Apaf1. Cyt *c* release is controlled through the Bcl-2 family, whereas the death-receptor extrinsic pathway is activated by death receptor ligands. HCMV infection was associated with increased cellular levels of antiapoptotic Bcl-2 and Bcl-X_L. Cyt *c* release is inhibited through stabilization of the mitochondrial membrane by vMIA (pUL37x1). vICA (pUL36) inhibits cleavage of procaspase-8 in active caspase 8. HCMV IE2-86 stimulates expression of death receptor ligands on the surface of infected cells. HCMV UL144 is hypothesized to encode for a soluble decoy receptor for TNF-α. Apaf1, apoptotic protease-activating factor1; Cyt *c*, cytochrome *c*; FADD, Fas-associated death-domain; TNF-α, tumor necrosis factor-α; vICA, viral inhibitor of caspase activation; vMIA, viral mitochondrial inhibitor of apoptosis.

HCMV infection and the intrinsic apoptosis pathway

In response to diverse cytotoxic agents, apoptosis usually occurrs via the intrinsic apoptosis pathway. Through this pathway, reduced mitochondrial membrane integrity results in release of mitochondrial cytochrome *c*, to the cytoplasm, followed by association of mitochondrial cytochrome *c* with Apaf-1 and ultimately with procaspase-9 to form the apoptosome. This results in activation of caspase-9 and of downstream caspases such as caspase-3. The Bcl-2 protein family that consists of anti-apoptotic (e.g. Bcl-2, Bcl-X_L, Bcl-w, A1 and Mcl-1) and pro-apoptotic (e.g. Bax, Bak, Bad, Bik, Puma, Noxa and Bid) members plays a central role in the regulation of mitochondrial membrane integrity and therefore in control of the intrinsic apoptotic pathway. However, the exact mechanism of action of Bcl-2 family proteins is not yet fully understood. Whereas antiapoptotic members of this family stabilize mitochondrial membrane and therefore inhibit cytochrome *c* release from mitochondria, pro-apoptotic members destabilize mitochondrial membranes and facilitate release of cytochrome *c* and other pro-apoptotic proteins [37–39]. Bcl-2 and its closest pro-survival relatives, Bcl-X_L and Bcl-w, directly associate with the mitochondrial outer membrane and the endoplasmatic reticulum/nuclear membrane to maintain their integrity [37]. A group of other pro-apoptotic family members, such as Bax and Bak, closely resemble Bcl-2, especially

in Bcl-2 homology (BH) domains, including BH1, BH2 and BH3, but how they interfere with Bcl-2 in detail remains uncertain. It has been postulated that Bax and Bak oligomerize in the mitochondrial outer membrane, breaching its integrity and resulting in the release of cytochrome *c* and other pro-apoptotic proteins and ultimately causing activation of caspase-9 [38, 39]. The other pro-apoptotic proteins, such as Bid, Noxa and Puma, have only the short BH3 motif. These BH3-only proteins (with the possible exception of Bid) act as damage sensors and direct antagonists of the pro-survival proteins of the Bcl-2 family [37], but cannot cause cell death in the absence of Bax and Bak. An increase in anti-apoptotic Bcl-2 family members leads to increased resistance of cells to apoptosis caused by various stimuli. This may lead to cell survival despite DNA damage, which compromises genomic stability and supports further mutations of tumor cells [37, 40–42].

HCMV interferes with the intrinsic apoptosis pathway in different ways (fig. 1). Increased expression of Bcl-X_L in HCMV-infected endothelial cells suggest that HCMV infection might protect cells from apoptosis by Bcl-X_L upregulation [43]. Moreover, HCMV infection induces alteration of apoptosis and drug resistance in some tumor cells at least in part due to upregulation of Bcl-2 family proteins. Increased Bcl-2 protein levels have been demonstrated in HCMV-infected colon tumor cells [2]. Neuroblastoma cells persistently infected with HCMV revealed an upregulation of Bcl-2 and a decreased sensitivity of infected cells to the cytotoxic drugs etoposide and cisplatin, as compared with noninfected cells [16]. Treatment with ganciclovir not only stopped HCMV replication but also decreased Bcl-2 protein levels and restored sensitivity to both anticancer drugs and inducibility of apoptosis to the levels of non-infected cells [16]. These two reports suggest that HCMV infection induces alteration of apoptosis and drug resistance in certain tumor cells, at least in part due to upregulation of Bcl-2 protein.

In addition to interactions with cellular members of the Bcl-2 family, HCMV may influence intrinsic apoptosis via a newly identified viral mitochondrial inhibitor of apoptosis (vMIA), a gene product of [UL37 ORF pUL37 exon 1 (pUL37x1)] [44, 45]. vMIA is a type-1 heavily glycosylated transmembrane protein localized primarily in the mitochondria following HCMV infection that inhibits mitochondrial megapore activation in a manner similar to that of members of the anti-apoptotic Bcl family [44]. In mitochondria vMIA forms a complex with adenine nucleotide translocator, which is believed to be a component of the mitochondrial transition pore complex [46], and thus suppresses apoptosis by blocking permeabilization of the mitochondrial outer membrane. This is the only UL37 function that is essential for viral replication in cultured cells, and is a leading candidate for prevention of virus-induced, intrinsic apoptosis. HeLa cells expressing vMIA were resistant to apoptosis triggered by doxorubicin [44]. In addition to vMIA, two longer splice variant products of the UL37 gene, gpUL37 and gpUL37$_M$, were recently identified. Both gpUL37 and gpUL37$_M$ are dispensable for virus replication in cultured cells as long as vMIA is present. Their biological function remains undetermined and may be unrelated to their anti-apoptotic activity [44, 47]. Despite the similarity of behavior to anti-apoptotic members of the Bcl-2 family, vMIA does not share any significant amino acid sequence homology with Bcl-2, and unlike Bcl-2 or Bcl-X_L, it does not bind proapoptotic Bcl family members or voltage-dependent anion channels. These structural and functional differences between vMIA and Bcl-2 suggest that vMIA represents a separate class of cell death suppressors. Interestingly, vMIA is a broadly acting inhibitor that can suppress apoptosis not only induced by stimuli of intrinsic apoptosis but also by stimuli of death receptor pathway [44]. The mechanism of anti-apoptotic action of vMIA is not fully elucidated; however, recently the association between vMIA expression and disruption of the reticular mitochondrial network in HCMV-infected fibroblasts was revealed. vMIA altered the fission and/or fusion process that normally controls mitochondrial networks. Although mitochondrial fission usually is associated with induction of apoptosis, vMIA caused mitochondrial disruption and inhibited apoptosis subsequent to this event [45].

HCMV infection and p53

The cellular response to DNA damage is commonly regulated by p53, although p53-independent apoptosis pathways are also known [40, 48, 49]. In response to DNA damage, p53 may initiate either apoptosis or cell cycle arrest and repair. The response depends on the cell type and the cause of DNA damage [50, 51]. HCMV 86-kDa immediate early protein 2 (IE2-86) was shown to bind to p53 and suppress its transactivating function, which is important for induction of apoptosis [52, 53]. Overexpression of IE2-86 prevents doxorubicin-induced apoptosis in smooth muscle cells [54] and p53-dependent apoptosis caused by nonpermissive temperature, while contributing had no influence on ultraviolet (UV) light-induced apoptosis [55].

In addition to direct suppression of p53 action by IE2-86, HCMV contains in vitro transforming oncoproteins such as morphologic transforming regions (mtr), i.e. mtrI, mtrII and mtrIII. These genes may in part be responsible for the tumorigenic phenotype observed in some human cancers; however only mtrII (UL111A) is retained and expressed in both transformed and tumor-derived cells and is required for maintenance of the transformed phenotype. The transforming and tumorigenic activities of

the mtrII oncoprotein are localized to an open reading frame (ORF) encoding a 79-amino acid (aa) protein. The mtrII protein binds to p53 and inhibits its ability to transactivate a p53-responsive promoter [56, 57].

Induction of ΔNp73 by HCMV

p73 is a close homolog of p53. It is expressed in different isoforms depending upon the mode of splicing of messenger RNA (mRNA) and may also be involved in apoptosis inhibition by HCMV infection. The p73 isoforms induce p53-responsive promoters by their transactivating domain (TA), thereby causing growth arrest or triggering apoptosis similar to p53 [58, 59]. Exogenously expressed p73 induces both morphological (neurite outgrowth) and biochemical (expression of neurofilaments and NCAM, downregulation of N-myc, upregulation of pRB) markers of neuronal differentiation in neuroblastoma cell culture that are associated with decreased malignant behavior [60]. Moreover, one report reveals that p53 requires p73 and p63/p51 (the third family member) to induce DNA-damaged apoptosis, indicating a very tight relationship between the three members of the same family [61]. Despite similar sets of target genes, a number of biological differences exist between p53, p73 and p63. Unlike p53, the p73 and p63 genes do not represent 'bona fide' tumor suppressor genes, and tumors rarely contain mutations in p73 and p63. The p73 gene was found to contain a second transcriptional start site, giving rise to the expression of ΔNp73, a species of p73 protein that lacks the N-TA domain. ΔNp73 is a dominant-negative inhibitor of p53 and p73 that was found to be overexpressed in cell lines from breast cancer gynecological cancers and neuroblastic tumors [59, 62]. Moreover, ΔNp73 overexpression was shown to facilitate immortalization of primary cells and to cooperate with oncogenic Ras in transformation in vitro and in vivo [63]. It efficiently counteracts transactivation function, apoptosis and growth suppression mediated by p53 and p73 and induces drug resistance in p53-positive tumor cells [58, 59, 62]. In neuroblastoma patients, ΔNp73 expression represents a prognostic marker for poor outcome [64]. Recent observations demonstrate that HCMV infection induces robust accumulation of ΔNp73 α isoforms through virus-induced protein stabilization [65]. HCMV-induced ΔNp73 α exerted a dominant negative effect on both p73 α- and p53-dependent apoptosis. Anti-apoptotic effects of HCMV in both p53-negative tumor glioblastoma U373MG cells and neuroblastoma IMR-32 cells expressing functional p53 are in line with this [66]. Although specific viral components inducing elevated ΔNp73 levels have not been identified yet, the levels of ΔNp73 were upregulated as early as 4 h post infection, suggesting that viral replication is not obligatory for observed effects. Expression of ΔNp73 correlated with poor outcome in neuroblastoma patients [64], suggesting that HCMV may promote progression of neuroblastoma due to induction of ΔNp73. Moreover, HCMV stimulates expression of N-myc in neuroblastoma cells [15, 17], and N-myc suppresses p73 expression in neuroblastoma cells [67]. In addition to upregulation of N-myc, HCMV also upregulates c-myc in various cell types [68–70], which is also known to inhibit p73 activity [71]. Thus, suppression of p73 by HCMV-caused upregulation of N-myc and/or c-myc and induction of ΔNp73 might act together in inhibition of apoptosis and differentiation in HCMV-infected tumor cells (fig. 2).

HCMV infection and MAPK signaling

The mitogen-activated protein kinase (MAPK)-signaling cascades are involved in regulation of cellular response to exogenous factors, including geno- and cytotoxic cancer treatments [72]. The extracellular stimulus-regulated kinases 1 and 2 (ERK1/2), the stress-activated/c-Jun NH_2-terminal protein kinases (SAPK/JNK) with their three family members (JNK 1, JNK 2, JNK 3), and the four p38 enzymes (p38α, p38β, p38χ, p38δ) are involved in the response to cytotoxic treatment such as anticancer chemotherapy or radiation therapy.

The Ras/Raf/MEK/ERK signal transduction cascade is a vital mediator of a number of cellular fates, including growth, proliferation, and survival, and may be involved in the oncogenic behavior of tumors. Phosphorylation of the ERKs mainly occurs as a result of growth factor/cy-

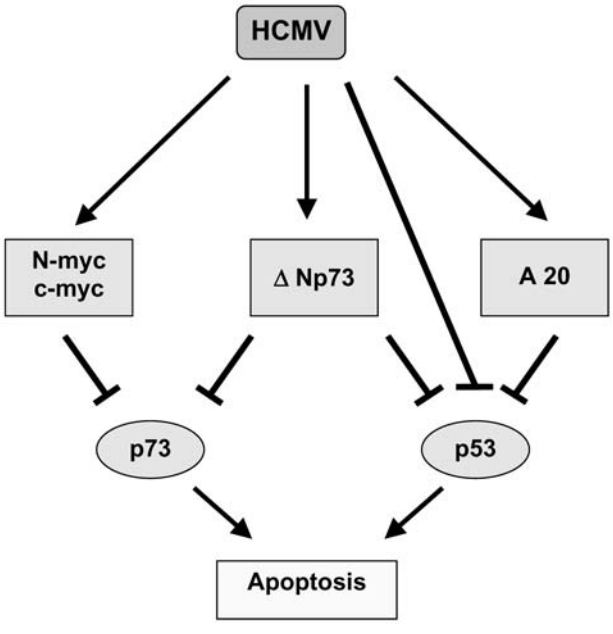

Figure 2. HCMV inhibits p53- and p73-induced apoptosis by direct and indirect mechanisms.

tokine stimulation, but was also shown to be activated by hydrogen peroxide, UV light and ionizing radiation [73, 74]. Expression of significant levels of phosphorylated ERK1/2 was found in cell culture of different tumors [75]. This aberrant activation of ERK1/2 is implicated in the growth and pathologic behavior of cancer cells [76]. Activating mutations of Ras, Raf and MEK are able to oncogenically transform fibroblasts in vitro [77–79]. The activation of MEK/ERK may protect cells against Fas-induced apoptosis and may be responsible for TPA-related drug resistance [74]. Activation of the Raf/MEK/ERK pathway promotes growth and inhibits apoptosis in hematopoietic cells. The combination of MEK inhibitors with radiation or chemotherapeutic agents result in synergistic cytotoxic effects [80]. ERK1/2 activate the transcription factor AP-1, which promotes survival of cells with proliferative capability [81–83]. Data demonstrating that HCMV infection results in the maintenance of previously activated ERK1/2 [84] suggest that HCMV infection of tumor cells might be able to induce cell survival through ERK1/2 signaling. Moreover, activation of ERK1/2 appears to be important for HCMV replication, because inhibition of the MEK/ERK-pathway decreases HCMV replication. These results are in accord with investigations demonstrating that HCMV IE1 activates AP-1 under involvement of the MEK/ERK pathway [85]. Therefore, HCMV infection is likely to cause activation of the cellular MEK/ERK pathway, which in turns contributes to apoptosis inhibition by HCMV.

HCMV infection and Akt (PKB) signaling

The cellular kinase Akt (also known as protein kinase B, PKB), a cellular homolog of the oncoprotein of AKT8 retrovirus (for reviews see [86, 87]), is activated by phosphatidylinositol-3 kinase (PI-3K) in response to many factors, including insulin and insulin-like growth factor (IGF) [86]. Initially, prior to its identification as a general mediator of cell survival, Akt was found to be necessary for the maintenance of glucose homeostasis. Dominant negative alleles of Akt suppress IGF-1-mediated cell survival, and constitutively active Akt prevents PTEN-mediated apoptosis [87]. Akt phosphorylates a number of different proteins involved in the cell death machinery and thus interferes with apoptotic cascades. It phosphorylates the pro-apoptotic Bcl-2 family member Bad, which forms heterodimers with anti-apoptotic Bcl-X_L. Phosphorylation of Bad inhibits formation of Bad/Bcl-X_L heterodimers and therefore maintains the anti-apoptotic activity of Bcl-XL [88]. Moreover, Akt represses the catalytic activity of caspase 9 by phosphorylation [89]. It also phosphorylates FKHR, a member of the Forkhead family of transcription factors, and prevents its nuclear translocation and the activation of FKHR gene targets, which include several pro-apoptotic proteins, such as BIM and FasL [90].

The PI-3K pathway may be activated by HCMV infection in different ways [91, 92]. Viral proteins and/or components of virus structure appear to activate Akt. HCMV IE1-72 and IE2-86 proteins induce PI-3K activation, which in turn leads to Akt activation. Akt activation could inhibit apoptosis in temperature-sensitive ts13 cell lines grown at the nonpermissive temperature [93]. Moreover, a recent report demonstrates that HCMV initiated infection and intracellular signaling by interaction with the epidermal growth factor receptor (EGFR) [92]. HCMV binding results in EGFR phosphorylation and downstream activation of the PI-3K pathway followed by Akt phosphorylation. Moreover, Akt can also modulate cell survival by indirect effects on two central regulators of cell death – nuclear factor κB (NF-κB) and p53 [87]. Akt can phosphorylate and activate the inhibitor of NF-κB (IκB) kinase (IKK), which induces degradation of IκB and, therefore, increases NF-κB stability [92, 94]. This mechanism is in concordance with the very rapid activation of NF-κB in a protein synthesis-independent manner, which was shown by different groups, including our own [95, 96]. NF-κB has already been associated with suppression of apoptotic cascades [97]. In addition to the described activation via the PI-3K pathway, HCMV proteins such as IE1 are able, at least in part, to activate NF-κB molecules directly [98]. In addition, NF-κB may be activated via interaction of HCMV glycoproteins with the pattern recognition receptors Toll-like receptor 2 and CD14 [99]. Moreover, possibly through activation of Akt and NF-κB, HCMV induces expression of another immunoregulatory gene, A20 [100], which protects cells from p53-mediated apoptosis [101–103] (fig. 2). Possibly, these different mechanisms work together in NF-κB activation and, therefore, in NF-κB-mediated apoptosis inhibition by HCMV.

In tumor cells, HCMV-induced Akt activation may be important for promoting malignant behavior because Akt-induced metabolic changes may be of benefit for tumor cell growth and inhibition of apoptosis [87]. For example, activation of Akt was shown to play an important role during the progression of colorectal carcinoma [104], which is frequently associated with HCMV infection. In addition to facilitating cell growth and rescue tumor cells from apoptosis, Akt phosphorylation is associated with a higher grade of clinico-pathological parameters of malignancies, including depth of invasion, infiltration to venous vessels, lymph node metastasis and clinico-pathologic stage [104].

Therapeutical implications and perspectives

Given an antiapoptotic role of HCMV infection in tumor progression, the impact of HCMV on specific malignan-

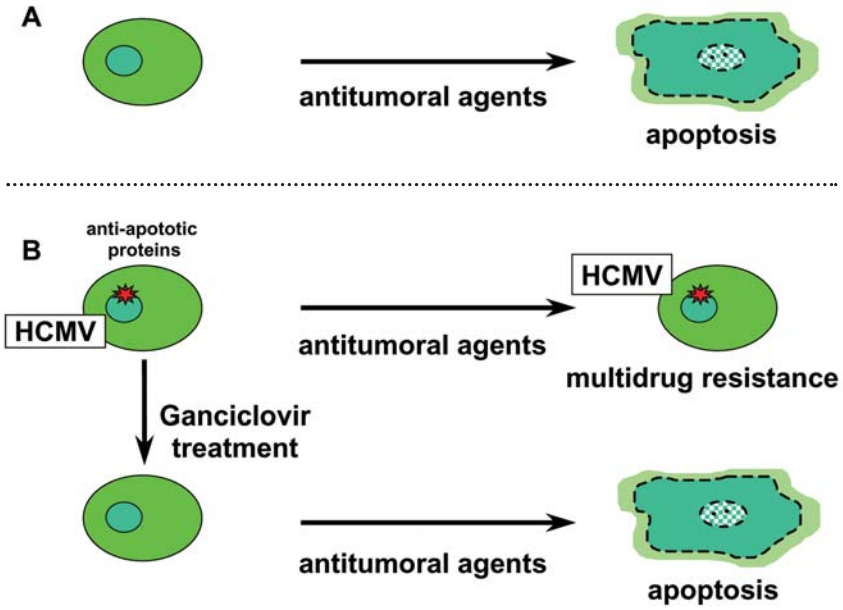

Figure 3. Model for the action of antiviral therapy for the treatment of HCMV-infected tumor cells. Ganciclovir inhibits HCMV replication and anti-apoptotic effects, thereby restoring sensitivity to antitumoral treatment.

cies should be validated in prospective clinical trials. Since HCMV may influence the course and prognosis of specific cancers [1–14], the detection of viral DNA or antigens in tumor tissues may become a novel prognostic marker for cancer patients and may influence treatment strategy.

In fact, our preclinical model showed that treatment of HCMV-infected tumor cells with the anti-HCMV drug ganciclovir, abolished virus production, reestablished sensitivity to chemotherapy, lowered Bcl-2 expression and facilitated inducibility of apoptosis to the level of the parental cells [16] (fig. 3). In persistently infected cultures both infected and noninfected cells were protected against apoptosis. This is in concordance with observations, showing that drug-resistant cells are able to influence the phenotype of surrounding nonresistant cells [105]. Although the therapeutical relevance of these findings remains to be clarified, the strong anti-apoptotic effects of virus in persistently infected tumors make an intensive investigation of the disease outcome of HCMV-infected tumors necessary.

In this manuscript we discussed numerous mechanisms by which HCMV exhibits antiapoptotic effects. Their relevance must be confirmed in animal models using tumor cells persistently infected with HCMV or expressing viral antiapoptotic proteins. This may facilitate understanding of the role of HCMV infection for metastatic behavior of tumors and their sensitivity to cytostatic treatment. Yet increased metastasis formation of persistently HCMV-infected neuroblastoma cells relative to non-infected tumors was observed [15].

Conclusions

The ability of HCMV to preferentially infect tumor tissues suggests a unique character of mutual interaction between the metabolism of tumor cells and HCMV. The interaction of HCMV with host tumor cells may rescue tumor cells from apoptosis. This is of special interest for cancer therapy, since apoptosis inhibition is considered to be a major reason for chemo- and radiotherapy failure. Therefore, new insights could improve diagnosis of HCMV-associated malignancies and influence the choice of therapy. Further insight into the molecular mechanisms of HCMV-mediated apoptosis inhibition as well as knowledge of the clinical consequences is necessary. The ability of antiviral therapy to reverse the resistance of HCMV-infected tumor cells encourages investigation of antiviral drugs as part of the treatment regimen for patients with HCMV-infected tumors.

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