BCL-2 mediated suppression of cell death promotes hypoxia-induced tumor angiogenesis in human pancreatic cancer cells

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Abstract

Increased expression of BCL-2 has been linked to increased pathogenesis in many systems including our own observations in isogeneic metastatic pancreatic carcinoma cell lines. The fundamental effect of BCL-2 is to prevent apoptotic and necrotic cell death. BCL-2 may have additional effects on the cellular level, which may influence overall tumorigenicity. We investigated the link between BCL-2 and the process of angiogenesis.

We transfected human pancreatic adenocarcinoma cell line MiaPaCa-2 with human BCL-2 and investigated its effect on the growth of intrapancreatic tumors following orthotopic injection of $1 \times 10^6$ tumor cells into the pancreas of nude mice. Primary pancreatic tumor samples were harvested 23 days after tumor cell injection. By immunohistochemistry, we determined cell proliferation (Ki67), cell death (DNA fragmentation, TUNEL), microvessel density (CD31), and tumor cell produktion of vascular endothelial cell growth factor (VEGF). By electrophoretic motility shift assay (EMSA) and confocal microscopy we evaluated in vitro the transcriptional activity of NFκB (nuclear factor kappa B) and HIF-1α/ARNT (hypoxia inducible factor) in human pancreatic cancer cells following stimulation by hypoxia.

Results: Tumors derived from three different BCL-2 transfectants revealed marked increase in tumor weights. However, despite their massive size histological examination showed virtually no central necrosis. Furthermore, TUNEL staining for DNA fragmentation in situ revealed sharply reduced levels of cell death consistent with the known effects of BCL-2. Proliferation was enhanced as measured by PCNA staining. Tumors from BCL-2 tumors were stained with CD-31, an endothelial cell antigen, and blood vessels were quantified. Both mean microvessel density and size were significantly increased in BCL-2 tumors. These effects were associated with enhanced VEGF staining. In vitro exposure of the transfectants to hypoxia confirmed their ability to protect from cell death. The VEGF production in the BCL-2 transfectants was slightly elevated than in the controls. However, hypoxia induced VEGF production was markedly higher at both the mRNA and protein levels. One candidate for hypoxia induced VEGF production is HIF-1α/ARNT. Confocal microscopy and electrophoretic motility shift assays (EMSA) demonstrated that the parental cell line exhibited extensive nuclear localization of ARNT. Surprisingly, the
transfectants exhibited no nuclear localization of ARNT. Therefore we investigated the role of NFκB that it plays in cell survival and angiogenesis. Confocal microscopy and EMSA confirmed that hypoxia induced an increase in nuclear localization of p65Rel protein in BCL-2 transfectants. Stable transfection of parental lines with dominant active inhibitor of NFκB (IkB) completely blocked both basal and hypoxia induced VEGF expression confirming that BCL-2 mediated NFκB activation was required.

Our results indicate that BCL-2 may have additional effects on tumor pathogenicity beyond its anti-apoptotic functions. In fact, it may aid in regulating other factors that may enhance tumor survival. We have demonstrated that increased levels of BCL-2 in pancreatic carcinoma cell lines dramatically effects transcription factor activity leading to upregulation of VEGF under hypoxic conditions and a subsequent increase in microvessel density in tumors. These results provided additional insight into the effects of increased expression of BCL-2 in tumors.

Einleitung

Der fundamentale Effekt von BCL-2 ist es, den apoptotischen und nekrotischen Zelluntergang zu verhindern [1, 2]. Klinische und immunhistochemische Untersuchungen haben ein signifikant schlechteres Überleben der Patienten mit Adenokarzinom des Pankreas gezeigt, in deren Tumorpäparate immunhistochemisch eine starke Anreicherung von BCL-2 nachgewiesen werden konnte. Sauerstoffminderzversorgung (Hypoxie) ist neben Azidose einer der Schlüsselregulatoren für Angiozeugenese [3], und induziert einen Komplex von Signaltransduktionsvorgängen, unter anderem die Aktivierung und Stabilisierung des Transkriptionsfaktors, hypoxia-inducible factor-1α (HIF-1α) [4].

Unser Ziel war es, die Interaktion zwischen BCL-2 Expression und Angiogenese beim Pankreaskarzinom zu bestimmen.

Material und Methoden

Humane Pankreaskarzinomzellen (MiaPaCaII) wurden mit humanem BCL-2 transfiziert und anschliessend orthotop in Nacktmäuse injiziert (1 × 10⁶). Immunohistochemisch wurde Zellproliferation (Ki67), Zelluntergang (DNA Fragmentation, TUNEL), Gefässdichte (microvessel density, CD31) und Produktion von vascular endothelial cell growth factor (VEGF) gemessen. Durch electrophoretic motility shift assay (EMSA) und konfocale Mikroskopie wurde die Aktivität der Transkriptionsfaktoren NFκB und HIF-1α/ARNT gemessen.

Ergebnisse

23 Tage nach ortotoper Tumorzellinjektion wurde ein signifikanter Unterschied im Tumorgewicht zugunsten der BCL-2 transfizierten MiaPaCaII-Zellen festgestellt (1.5 – 2.5 g vs. 0.4 g). Makroskopisch waren die Tumoren ausgehend von BCL-2 transfizierten MiaPaCaII Zellen wesentlich vascularisierter und zeigten keine zentralen Nekrosen im Vergleich zu den Parentalzelltumoren. Immunohistochemische Analysen der Tumor-