Role of inhibitor-of-apoptosis protein livin (BIRC7) in neuroblastoma:
Prognostic and functional significance

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ABSTRACT

Purpose: Livin (BIRC7, ML-IAP) is a recently identified member of the inhibitor-of-apoptosis (IAP) family of anti-apoptosis proteins, and its expression has been reported to have potential prognostic significance in several malignant tumors. The aim of this study was to evaluate livin gene expression in neuroblastoma by quantitative RT-PCR in order to assess the prognostic relevance of livin and to develop a clinically useful, quantitative assay for livin expression in neuroblastoma biopsies. We further examined the role of livin in protecting neuroblastoma cells from chemotherapy-induced and spontaneous apoptosis.

Experimental design: A total of 59 neuroblastoma tumors were evaluated. Livin gene and protein expression were determined by qRT-PCR and immunoblot, respectively. The qRT-PCR results were compared to various clinical and pathological features associated with neuroblastoma by the Kruskal-Wallis test and correlated with survival by the Cox proportional hazard model. The functional role of livin was investigated by overexpressing livin ectopically and by repressing livin using siRNA. Sensitivity to chemotherapeutic drugs and apoptosis were measured by WST and nucleosome-release assays, respectively.

Results: Livin gene expression was high in 39%, intermediate in 49%, and low in 12% of cases studied. Livin protein expression was confirmed by immunoblot. Livin expression alone did not correlate with patient survival; however, patients with high livin gene expression and amplified MYCN had significantly decreased survival compared to patients lacking one or both markers (Log-rank test, p = 0.01). Livin-transfected neuroblastoma cells showed increased resistance to doxorubicin and etoposide but not to vincristine and paclitaxel. Conversely, livin knockdown sensitized neuroblastoma cells to doxorubicin and induced spontaneous apoptosis in the absence of chemotherapeutic agents.

Conclusion: We identified high levels of livin gene expression as a biological risk factor that significantly worsens treatment outcome for patients with MYCN-amplified neuroblastoma. We also confirmed the utility and relevance of qRT-PCR for evaluating livin expression clinically. Our data further suggest that livin protects neuroblastoma
cells from genotoxic agents and from spontaneous apoptosis, supporting the notion that livin may be a potential therapeutic target.

**Clinical Relevance:** *MYCN*-gene amplification is one of the strongest predictors of poor prognosis in neuroblastoma, a common tumor of childhood. Our data suggest that elevated expression of livin in neuroblastoma cells with amplified *MYCN* confers an especially poor prognosis, and therefore livin expression may represent a novel risk factor when combined with *MYCN*-amplification. Additionally, our results indicate that qRT-PCR provides a clinically useful technique for determining livin expression in tumor biopsies and further suggest that livin itself may be an important target for neuroblastoma therapy.