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Defective NF-κB Signaling in Metastatic Head and Neck Cancer Cells Leads to Enhanced Apoptosis by Double-Stranded RNA

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Abstract

Ligands to several Toll-like receptors (TLR), which mediate innate immune responses and chronic inflammation have been used as adjuvants to immunotherapy to enhance their antitumor activity. In particular, double-stranded RNAs that are cognate ligands of TLR3 have been used to trigger proapoptotic activity in cancer cells. However, a mechanistic understanding of TLR3-mediated apoptosis and its potential involvement in controlling tumor metastasis has been lacking. In this study, we used paired cell lines and fresh tumor specimens, derived from autologous primary and metastatic head and neck squamous cell carcinoma, to investigate the role of TLR3 signaling in metastatic progression. Compared with primary tumor cells, metastatic tumor cells were highly sensitive to TLR3-mediated apoptosis after double-stranded RNA treatment. Enhanced apoptosis in metastatic cells was dependent on double-stranded RNA and TLR3 and also the TLR3 effector signaling protein TRIF. Downstream responses requiring NF-κB were critical for apoptosis in metastatic cells, the defects in which could be resuscitated by alternative pathways of NF-κB activation. By elucidating how TLR3 ligands trigger apoptosis in metastatic cells, our findings suggest insights into how to improve their clinical use. Cancer Res; 72(1); 45–55. ©2011 AACR.

Introduction

Chronic inflammation has been established as a hallmark of tumorigenesis (1–3). Owing to their ability to induce proinflammatory cytokines, Toll-like receptor (TLR) and other innate immune receptor signaling pathways in the context of tumor initiation, progression, and metastasis have attracted close attention in recent years. Although the role of chronic inflammation in tumor initiation is well accepted, a more complex picture has emerged for tumor metastasis (4, 5). Several TLR agonists have shown anticancer activities, whereas others promote tumorigenesis (6, 7). This apparent complexity may be related to the 2 primary signaling pathways (17–19). In most cases, activation of NF-κB leads to induction of proinflammatory cytokines such as interleukin (IL)-1β, TNF-α, and IL-6. On the contrary, activation of IRFs causes induction of IFN and IFN-stimulated genes with anti-growth properties. Depending on the cell type, some TLRs preferentially activate proinflammatory NF-κB, whereas others promote antigrowth-promoting IRFs.

Unique among the TLRs, TLR3—a sensor for double-stranded RNA (dsRNA), uses the adaptor protein TRIF (TIR domain-containing adapter inducing IFN-β) to activate both IFN3 and NF-κB signaling pathways (8). Activation of these transcription factors drives the downstream gene induction, including IFN-β, several IFN-stimulated genes (ISG), and IκBα (9). As a strong inducer of type I IFN, TLR3 activation has been shown to cause growth arrest and apoptosis in cancer cells (10–16). Besides TLR3, activation of other dsRNA sensors, RIG-I and MDA5, has been shown to cause apoptosis by multiple signaling pathways (17–20). Beyond apoptosis, the synthetic dsRNA polyinosinic-polycytidylic acid [poly(I):poly(C)] has been found to be highly effective as adjuvant (21). Indeed, in vivo evidence regarding the anticancer role of TLR3 has come from a number of studies. Chin and colleagues used TRAMP (transgenic adenocarcinoma of mouse prostate) model and showed TLR3 null mouse to be more prone to tumor development and progression (22). Salaun and colleagues reported decreased relapse following dsRNA treatment in TLR3-positive breast cancers (23).

Head and neck squamous cell carcinomas (HNSCC) are the most frequent tumor types in the upper aerodigestive tract (24, 25). Activation of TLR4 and the NF-κB pathway has been shown to promote HNSCC development as well as proliferation (26, 27). On the contrary, activation of TLR3 has been shown to induce apoptosis (28). Nodal metastasis is the most important prognostic factor in HNSCC, and inflammatory signals in the
mucosal and nodal environment may promote tumor invasion, survival, and metastasis leading to treatment resistance (29, 30). To understand the role of TLR3 signaling in metastatic progression of HNSCC and its potential immunotherapeutic role, we used well-characterized, autologous pairs of primary and metastatic HNSCC cell lines (31) and characterized their response to synthetic dsRNA poly(I):poly(C). We show that metastatic HNSCC cells manifest dramatically enhanced apoptosis in response to treatment with poly(I):poly(C) compared with the primary tumor cells mediated by the TLR3–TRIF signaling pathway. The enhanced apoptosis appears to be due to defective poly(I):poly(C)–mediated NF-κB activation in metastatic cells. For the first time, these results indicate specific sensitivity of metastatic cells toward poly(I):poly(C)–TLR3-mediated apoptosis and provide important potential for tumor-targeted therapy.

Materials and Methods

Cell lines
The primary and metastatic HNSCC cell lines (PCI-6A/B, PCI-15A/B, and UM-SCC-22A/B) were derived from the primary and metastatic lymph nodes and characterized at the University of Pittsburgh as described before (24). All 6 HNSCC cell lines were tested for their authenticity by cytogentic finger printing and human leukocyte antigen typing. All cell lines were cultured in Dulbecco’s Modified Eagle’s Medium (Lonza), containing 10% FBS and penicillin/streptomycin (Lonza). Immortalized normal human keratinocytes were maintained as described before (32). HEK293 cells and HEK293-derived stable cell lines, WI-161 cells have been described before (33). The above cell cultures were tested for mycoplasma on a regular basis to ensure that only mycoplasma-free cell lines were used in this study.

Antibodies and chemical reagents
Details of the antibodies, reagents, and other methods used in this study are given in the Supplementary Materials. Synthetic dsRNA analogue poly(I):poly(C) was purchased from GE Healthcare and dissolved in PBS before use. This is a mixture of high-molecular-weight (≤12 kD) dsRNA polymer with potent IFN induction capability (33, 34).

Quantification of caspase 3 and caspase 7 activity
HNSCC cells (2 × 10^4 cells) in 96-well plates were stimulated with poly(I):poly(C) (50 μg/mL). After stimulation, Caspase-Glo 3/7 Assay Kit (Promega) was used for caspases 3/7 activity as per the manufacturer’s instructions. MTT assay (Promega) was carried out as per manufacturer’s instructions.

RNA interference–mediated silencing
IRF3 knockdown in PCI-15B cells was achieved by transfection of shRNA targeting IRF3 or control short hairpin RNA (shRNA)–expressing plasmid (pcDNA3 IRF3 shRNA; ref. 35) using Lipofectamine 2000. Following selection with G418, a single-cell clone with maximum IRF3 knockdown was used for subsequent experiment. TRIF siRNA and negative control stealth siRNAs (50 nmol/L each; Invitrogen) were transfected with Lipofectamine RNAiMAX (Invitrogen), and cells were used 48-hour posttransfection. Stable IFNAR1 knockdown PCI-15B cells were prepared by packaging the IFNAR1-shRNA-pLKO.1 (Sigma-Aldrich) into lentivirus followed by infection and puromycin selection.

Tumor samples
Autologous, paired primary and metastatic HNSCC tissues were obtained directly after surgical excision, according to the Institutional Review Board–approved protocol UPCI 99-069 at the University of Pittsburgh. Tissue specimens were harvested and processed by enzymatic digestion into single-cell suspensions, as previously described (30), or snap frozen in LN2 within 30 to 60 minutes.

Results
dsRNA induces enhanced apoptosis in metastatic head and neck cancer cells
To characterize the TLR3 response and understand its contribution to metastatic progression, we treated autologous primary (designated with suffix A) and metastatic (suffix B) HNSCC cell lines PCI-6, PCI-15, and UM-SCC-22 with poly(I):poly(C). Unexpectedly, metastatic cells showed a marked increase in cell death. We investigated this phenomenon further using several standard apoptosis assays. Metastatic cells, PCI-6B, PCI-15B, and UM-SCC-22B showed more pronounced apoptosis as detected by higher levels of cleaved PARP (Fig. 1A), increased caspase3/7 activity in a poly(I):poly(C) dose-dependent manner (Fig. 1B) compared with the primary tumor–derived cells. Similar enhanced apoptosis was observed in metastatic cells using Annexin V–propidium iodide (Supplementary Fig. S1) assays. Despite enhanced apoptosis in metastatic cells, there were no significant differences between the overall cell viability between 2 types of cells treated with poly(I):poly(C) as measured by MTT assay (Supplementary Fig. S2A). However, normal keratinocytes did not exhibit apoptosis with poly(I):poly(C) treatment (Fig. 1C). We tested the response of HNSCC cells to several other TLR ligands. Although most of the HNSCC cells expressed TLR4, TLR7, and TLR8 (Supplementary Fig. S2B), the enhanced apoptosis was only seen in response to poly(I):poly(C), not with lipopolysaccharide (LPS) or R848 (Fig. 1D). These results indicated that the metastatic HNSCC cells undergo increased apoptosis in response to TLR3 activation.

Both primary and metastatic cancer cells express similar levels of TLR3 signaling components
Next, we compared the expression levels of different TLR3 signaling pathway components in these cells. As shown in Fig. 2A and B, all the paired A and B cells expressed comparable levels of TLR3 and TRIF mRNA and TLR3 protein (Fig. 2C and Supplementary Fig. S3). HEK293 cells transfected with corresponding cDNA constructs or control vectors were used as positive and negative controls in the quantitative real-time PCR (qRT-PCR) assays. Due to their robust difference in poly(I):poly(C)–mediated apoptosis, we focused primarily on...
the autologous PCI-15A/B cell lines for mechanistic studies. However, as discussed later, we have used other paired cells as well as surgically obtained cancer tissue specimens to confirm our findings.

Stimulation by dsRNA or virus can induce apoptosis either dependently or independently of IRF3-mediated gene induction (20). We found no appreciable difference in IRF3 protein levels between PCI-15A and PCI-15B cells (Fig. 2D). To investigate the role of IRF3 in the enhanced apoptosis of PCI-15B cells, we generated stable IRF3 knockdown PCI-15B-shIRF3 and PCI-15B-shCtrl cells using either IRF3 targeted or scrambled shRNA (35). These cells had substantially (~80%) reduced IRF3 expression levels (Fig. 2E, lanes 1 and 4) and showed reduced poly(I)poly(C)–mediated induction of ISG56, which is a direct target of IRF3 (Fig. 2E, lanes 2 and 3 and lanes 5 and 6). However, the apoptosis induction was not affected in PCI-15B-shIRF3 cells compared with the PCI-15B-shCtrl cells (Fig. 2E, lanes 2 and 3 and lanes 5 and 6; Fig. 2F). These results suggested that the enhanced apoptosis in metastatic PCI-15B cells could not be attributed to differences in expression of TLR3 signaling components, nor was it due to their differences in IRF3-mediated gene induction.

Enhanced apoptosis in metastatic PCI-15B cells is mediated via TLR3–TRIF–dependent signaling

Type I IFNs are known to cause apoptosis in various malignant cells (36). We compared the levels of IFN-α and IFN-β mRNA expression in PCI-15A and PCI-15B by qRT-PCR. As shown in Fig. 3A and B, both the cell lines induced very similar levels of IFN and other cytokines, such as CCL5 (Supplementary Fig. S4A). Furthermore, when PCI-15B cells were treated with poly(I)poly(C) in the presence of anti-IFNAR2–neutralizing antibody (37), there was no significant decrease in apoptosis (Supplementary Fig. S4B, lane 3 vs. lane 2), whereas ISG56 induction was inhibited under these conditions. In contrast, anti-IFN receptor antibody could inhibit IFN-induced apoptosis. (Supplementary Fig. S4B, lane 3 vs. lane 4). Finally, we used shRNA–mediated knockdown of IFNAR1 to ablate IFNAR1 expression in PCI-15B (Supplementary Fig. S4C), and showed that the enhanced apoptosis in PCI-15B cells was largely independent of IFN signaling (Fig. 3C and 3D). TLR3 and its adaptor TRIF has been shown to mediate apoptosis induced by dsRNA (10, 14, 38, 39). We investigated the contribution of TRIF by either functionally silencing TRIF using TRIF-siRNA (Fig. 3E) or by blocking TRIF–TLR3 interaction. PCI-15B cells were transfected with TRIF or control siRNA followed by poly(I)poly(C) treatment. As shown in Fig. 3E and F, TRIF silencing significantly reduced apoptosis in PCI-15B cells. In a separate experiment, TRIF activity was neutralized using a peptide specifically targeted against the TRIF–TLR3 interaction domain (29). Incubation of PCI-15B cells with the TRIF inhibitor peptide resulted in reduction of apoptosis (Supplementary Fig. S4D, lane 3), whereas it was unchanged in the presence of the control peptide (Supplementary Fig. S4D, lane 4).

Defective NF-κB signaling in PCI-15B

TLR3 activation by dsRNA leads to activation of both NF-κB and IRF3 signaling pathways (9). Because NF-κB activation is known to activate prosurvival pathways, we hypothesized that defective NF-κB signaling might skew this response toward greater apoptosis in PCI-15B cells. One of the initial hallmark steps in NF-κB activation is phosphorylation and proteosomal degradation of IκBα. Upon poly(I)poly(C) treatment, PCI-15A cells showed the expected pattern of IκBα degradation starting after 30 minutes, followed by its expected resynthesis and reappearance after 120 minutes (Fig. 4A).
Figure 2. TLR3 signaling components in HNSCC cells. TLR3 (A), TRIF (B) expression levels in HNSCC cells. qRT-PCR analysis of HNSCC cells along with HEK293 cells transfected with TLR3 or TRIF expression constructs as controls was carried out on total RNA as indicated. Following normalization of each sample with internal control RPL32, mRNA levels were expressed as fold expression with respect to untreated HEK293 samples (‘). Each bar represents mean and SD from triplicate samples. C, TLR3 protein expression levels in PCI-15A and PCI-15B. Cells were analyzed for TLR3 expression by flow cytometry as described in Materials and Methods. D, analysis of IRF3 protein expression levels by immunoblotting in PCI-15 cells along with HEK293 cells as controls. E and F, IRF3 knockdown does not reduce poly(I):poly(C)-mediated apoptosis of PCI-15B cells. Endogenous IRF3 expression was knocked down in PCI-15B cells using shRNA against IRF3. The control PCI-15B-shCtrl and the PCI-15B-shIRF3 cells were treated with poly(I):poly(C) as indicated. Cells were analyzed by immunoblotting for apoptosis (c-PARP), IRF3-dependent gene induction (ISG56), IRF3 levels (IRF3), and loading controls (Actin) E, as well as by caspase 3/7 cleavage assay (F). Ctrl, control; p(I):(C), poly(I):poly(C).
However, in PCI-15B cells, there was no detectable IκBα degradation after poly(I):poly(C) treatment. This indicated a possible block in NF-κB activation in PCI-15B cells. Next, we tested the capacity for NF-κB–mediated gene induction following poly(I):poly(C) treatment. Figure 4B shows the induction of the NF-κB–dependent gene IκBα by qRT-PCR in all the paired HNSCC cell lines. In each metastatic cell line, relative induction of IκBα was severely reduced compared with their primary counterparts. In the case of another NF-κB–dependent gene, IL-8, a marked difference in IL-8 mRNA induction was
observed (Supplementary Fig. S5A). PCI-15B cells expressed little IL-8 protein at baseline and lacked any induction of IL-8 after poly(I):poly(C) or other TLR ligand treatments (Supplementary Fig. S5B). These results indicate that PCI-15B cells harbored a defect in the NF-κB signaling pathway upstream of IκBα degradation. As a control, when PCI-15B cells were treated with other NF-κB–activating cytokines, such as IL-1β or TNF-α, IκBα degradation, as well as IL-8 induction, was readily observed (Fig. 4C and Supplementary Fig. S5A). In PCI-15A cells, poly(I):poly(C) caused IκBα degradation at 60 minutes, whereas IL-1β and TNF-α caused IκBα degradation at 30 minutes followed by expected resynthesis at 60 minutes. In PCI-15B cells, no IκBα degradation was observed, yet IκBα degradation was seen at 30 minutes with IL-1β and TNF-α treatment without any resynthesis. Caspase activation is known to degrade components of the NF-κB pathway (40, 41). To exclude the possibility that caspase activation by poly(I):poly(C) treatment did not block NF-κB activation and was subsequent to NF-κB activation, we examined IκBα degradation in the presence of pan-caspase inhibitor Z-VAD-FMK, which did not change the IκBα degradation pattern in PCI-15 cells (Fig. 4D). As expected, Z-VAD-FMK treatment did inhibit poly(I):poly(C)–mediated apoptosis in both cell lines (Supplementary Fig. S5C and S5D).

We measured nuclear translocation of NF-κB p65 and p50 subunits in PCI-15 cells after poly(I):poly(C) treatments (Fig. 5). Nuclear fractions from PCI-15A and PCI-15B cells were immunoblotted with anti-p65 and anti-p50 antibodies (Fig. 5A and B). Interestingly, nuclear translocation of p65 and p50 was detected after poly(I):poly(C) treatment in PCI-15A cells but not in PCI-15B cells. These observations were confirmed by immunofluorescence on the same PCI-15 cells (Fig. 5C and D). Together, these results showed that in metastatic PCI-15B HNSCC cells, TLR3 stimulation with poly(I):poly(C) fails to activate NF-κB signaling and NF-κB–mediated gene induction.

Enhanced apoptosis of metastatic cancer tissues with poly(I):poly(C)

To investigate the in vivo relevance of our findings of increased apoptosis in metastatic cancer cells with poly(I):poly(C), we compared apoptosis in autologous, paired primary, and metastatic tumor samples derived from HNSCC patients undergoing ablative surgical resection. Tumor samples from 3 previously untreated patients were collected after surgery from both primary and metastatic lymph nodes. Following removal of connective tissues, samples were carefully minced, enzyme digested, and resuspended in culture media. Tissue suspensions were incubated with 50 μg/mL poly(I):poly(C) overnight followed by Western blotting and qRT-PCR analysis for TLR3 and apoptosis pathway components. As shown in Fig. 6A, poly(I):poly(C) treatment of freshly isolated tumor tissues resulted in significant apoptosis as measured by cleaved PARP in both primary and metastatic tumor samples. However, the metastatic samples consistently showed enhanced apoptosis after poly(I):poly(C) treatment, similar to the results obtained with HNSCC cell lines (Fig. 1). When the same samples were tested for IRF3 and NF-κB–mediated gene induction, we found similar levels of ISG56 mRNA induction in both primary and metastatic tumors (Fig. 6B) showing the effectiveness of poly(I):poly(C) treatment and functional IRF3 signaling. However, as observed in PCI-15 cells (Supplementary Fig. S5A), metastatic samples had much lower levels of basal IL-8 mRNA induction.
indicating defective NF-κB signaling, which is further shown by an unexpected inhibition of IL-8 mRNA after poly(I):poly(C) treatments (Fig. 6C). These results provide crucial in vivo evidence validating the phenotypic findings observed using cultured HNSCC cell lines.

**Enhanced apoptosis in PCI-15B is due to defective NF-κB activation**

Genes induced by NF-κB have been linked to inflammation and cellular survival (42). We hypothesized that if the lack of NF-κB activation in PCI-15B cells after poly(I):poly(C) treatment was responsible for enhanced apoptosis, we should be able to modulate this phenotype by activating or inactivating NF-κB by other means. To test this hypothesis, we first used the NF-κB inhibitor BAY11-7082 to pretreat PCI-15 cells before incubating them with poly(I):poly(C). As shown in Fig. 7A, in the presence of BAY11-7082, primary tumor-derived PCI-15A cells showed characteristic induction of apoptosis (Fig. 7A, lane 3 and Supplementary Fig. S6A) and inhibition of p65 nuclear translocation (Fig. 7B) with poly(I):poly(C), similar to PCI-15B albeit at a lower level; however, there was neither any change in apoptosis (Fig. 7A, lane 7 and Supplementary Fig. S6A), nor p65 nuclear translocation (Fig. 7B) observed in PCI-15B cells. This indicated that the inability of PCI-15B cells to activate NF-κB led to enhanced apoptosis by poly(I):poly(C) and that a similar phenotype could be obtained in PCI-15A cells by NF-κB inhibition. Next, we attempted to rescue from apoptosis by activating NF-κB prior to TLR3 activation. Because PCI-15B cells showed significant IL-8 induction following IL-1β treatment (Supplementary Fig. S5A), we...
only in metastatic cells, poly(I):poly(C) was unable to activate prosurvival NF-κB signaling, which leads to enhanced apoptosis in these cells. The detailed mechanistic analysis shows a phenotype also observed in 2 other paired, autologous primary/metastatic cell lines, showing similar results. The reasons for this altered phenotype are under investigation but could be related to the desensitization of primary tumor cells exposed to dsRNA in the mucosal aerodigestive microenvironment, as opposed to the sterile nodal site of metastatic cells. Alternatively, it might be due to the development of tolerance to inflammatory stimuli in the metastatic cells in the lymph node.

Inactivation of proapoptotic pathway genes such as BAX, TP53, and DAPK, or activation of antiapoptotic genes, such as BCL2, NF-κB, and survivin, is almost always associated with metastasis because apoptosis provides a multistep barrier to metastasis. As a result, apoptotic resistance is believed to be acquired early in tumorigenesis and has been shown to be directly correlated with metastatic potential. In this light, our observation of ligand-specific inactivation of NF-κB in metastatic cells is surprising and counterintuitive. However, inactivation of NF-κB in tumor-associated macrophages has been reported (44, 45). The prevalent explanation for this has been that after colonization, the metastatic cells develop a more tolerogenic phenotype to inflammatory signals. In our case, it is partly ligand specific, because as shown in Fig. 4C it is unique to TLR3-mediated NF-κB response and does not affect IL-1β or TNF-α signaling. However, NF-κB-mediated gene induction by IL-1β or TNF-α, as measured by IL-8 induction, was significantly reduced in metastatic cells (Supplementary Fig. S5A, and data not shown). Thus, in the metastatic HNSCC cells NF-κB inactivation may provide them with important immune tolerance.

As noted before, engagement of TLRs may inhibit tumor growth or in some cases promote tumorigenesis (6, 7). The antitumor activity of TLR activation has been shown to be partly mediated by IFN (46). However, direct apoptosis by TLR activation has also been shown in a number of tumor cells. dsRNA-mediated apoptosis has been characterized in a number of cells, in most cases using well-characterized cell lines established from cancer tissues (20). However, for the first time, we compared this phenotype between paired primary and metastatic cancer cells and tissue samples. The primary purpose of dsRNA-mediated apoptosis has been thought to be protection of the organism from spreading virus infection. However, as we have shown in Fig. 1C, treatment of cells by applying poly(I):poly(C) to the medium did not induce apoptosis in primary (nontransformed) epithelial cells. Among the 2 primary molecular sensors of dsRNA, the mechanism involved in apoptosis via RIG-I/MDA5 and MAVS is better understood than TLR3. A combination of IRF3-mediated gene induction dependent, and independent, mechanisms has been proposed to be involved. For the TLR3 pathway specifically, the adaptor TRIF has been shown to be essential for the induction of apoptosis, but the involvement of IFN production has been more nuanced (14, 38, 39). However, the involvement of inactivated NF-κB has not been described before. In our analysis, we found that IFN production was not the major contributing factor for enhanced apoptosis (Fig. 3C), whereas
TRIF was essential (Fig. 3D). We initially focused on the positive contributing factors which were inducing enhanced apoptosis in PCI-15B cells. However, as it turned out, the difference was in the antiapoptotic NF-κB pathway, not in the proapoptotic signaling, which contributed to the overall outcome (Fig. 7E). This shows a very crucial role that the basal and specific signal-dependent NF-κB pathway plays in different stages of tumorigenesis. Novel target agents have become commonplace in oncologic therapy. Specific tumor-targeted drugs that can be applied to affect nodal metastases would have great therapeutic potential in HNSCC, and should be pursued in vivo using TLR3 agonists, if our novel findings are validated by further work. Furthermore, agents targeting the NF-κB pathway, such as the proteasomal inhibitor bortezomib, have shown clinical activity in some malignancies.

In summary, we describe a unique phenotypic difference between primary and metastatic HNSCC cells. The cells from metastatic tumors show excess apoptosis-mediated cell death in response to dsRNA. This phenomenon is ascribed to their inability to activate prosurvival NF-κB signaling pathway via TLR3. Our findings may provide useful insights in the use of dsRNA for cancer treatment.
Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

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