Ectopic Expression of X-Linked Lymphocyte-Regulated Protein pM1 Renders Tumor Cells Resistant to Antitumor Immunity

Tae Heung Kang1,2, Kyung Hee Noh1, Jin Hee Kim1, Hyun Cheol Bae1, Ken Y. Lin7, Archana Monie2, Sara I. Pai6, Chien-Fu Hung2,4, T.-C. Wu2,3,4,5, and Tae Woo Kim1

Abstract

Tumor immune escape is a major obstacle in cancer immunotherapy, but the mechanisms involved remain poorly understood. We have previously developed an immune evasion tumor model using an in vivo immune selection strategy and revealed Akt-mediated immune resistance to antitumor immunity induced by various cancer immunotherapeutic agents. In the current study, we used microarray gene analysis to identify an Akt-activating candidate molecule overexpressed in immune-resistant tumors compared with parental tumors. X-linked lymphocyte-regulated protein pM1 (XLR) gene was the most upregulated in immune-resistant tumors compared with parental tumor cells. Furthermore, the retroviral transduction of XLR in parental tumor cells led to activation of Akt, resulting in upregulation of antiapoptotic proteins and the induction of immune resistance phenotype in parental tumor cells. In addition, we found that transduction of parental tumor cells with other homologous genes from the mouse XLR family, such as synaptonemal complex protein 3 (SCP3) and XLR-related, meiosis-regulated protein (XMR) and its human counterpart of SCP3 (hSCP3), also led to activation of Akt, resulting in the upregulation of antiapoptotic proteins and induction of immune resistance phenotype. Importantly, characterization of a panel of human cervical cancers revealed relatively higher expression levels of hSCP3 in human cervical cancer tissue compared with normal cervical tissue. Thus, our data indicate that ectopic expression of XLR and its homologues in tumor cells represents a potentially important mechanism for tumor immune evasion and serves as a promising molecular target for cancer immunotherapy.

Cancer Res; 70(8); 3062–70. ©2010 AACR.

Introduction

Cancer immunotherapy has been reasonably successful in generating tumor-specific immune responses. However, in some cases, it is observed that the generation of tumor-specific immune responses does not translate into tumor regression in cancer patients (1). One potential explanation is that tumor cells with their aberrant gene expression can possibly influence and impair the immune system in many ways. A better understanding of the mechanisms of tumor immune evasion will serve as an important objective in the field of cancer immunotherapy.

To elucidate mechanisms of immune escape associated with immune resistance, we used an in vivo selection strategy using a previously developed human papillomavirus (HPV)-16 E7-expressing cancer cell line, called TC-1/P0, which has served as a preclinical tumor model for testing various E7-specific cancer immunotherapies (2, 3), including an HPV-16 E7-expressing vaccinia virus vaccine termed Vac-Sig/E7/LAMP-1. The vaccine encodes a fusion protein consisting of an endoplasmic reticulum signal sequence, HPV-16 E7 gene, and the transmembrane and cytoplasmic domains of lysosome-associated membrane protein-1 (LAMP-1; ref. 4). Using immune selection, we generated an immune-resistant clone that has high expression of MHC class I, called TC-1/P3 (A17; refs. 5, 6). The TC-1 P3 (A17) cells showed immune resistance to CTL-induced apoptosis in vitro and in vivo compared with parental TC-1 cells (5). In addition, the resistance of A17 tumor cells was shown to depend on prosurvival Akt signaling pathway, which was confirmed by a pharmacologic approach using various kinase inhibitors and using small interfering RNA (siRNA) targeting Akt (5). Thus, we have
successfully generated an immune-resistant tumor cell model [TC-1 P3 (A17)], thereby developing a system that would allow us to identify genes that may contribute to the Akt-mediated immune resistance to antitumor immunity induced by various cancer immunotherapeutic agents.

In the current study, we have used microarray gene analysis to identify candidate molecules that are overexpressed in A17 tumors compared with P0 tumors. Our data indicate that the expression of X-linked lymphocyte-regulated protein pM1 (XLR) and its homologues in tumor cells represents a new mechanism of immune resistance via activation of the Akt pathway and has important implications for the development of a novel therapeutic strategy against immune-resistant tumor cells.

Materials and Methods

**Mice.** Six- to 8-wk-old female C57BL/6 mice and nude mice were purchased from Daehan Biolink. All animal procedures were done in accordance with recommendations for the proper use and care of laboratory animals.

**Microarray data analysis.** For the microarray analysis, the RNA samples from TC-1 and TC-1 P3 (A17) cell lines were prepared and analyzed with Affymetrix GeneChip Mouse Genome 430 2.0 arrays. To estimate the gene expression signals, image analysis was done for the CEL files of the chips using the statistical technique and package guanine-cytosine content–robust multiarray analysis as described previously (6, 7). The normalized signal intensities were analyzed using parametric empirical Bayes method to estimate the posterior probabilities of differential expression of genes between TC-1 and TC-1 P3 (A17) cell lines (8, 9).

**DNA constructs and siRNA transfection.** For the generation of the pMSCV construct encoding XLR, synaptosomal complex protein 3 (SCP3), XLR-related, meiosis-regulated protein (XMR), or human SCP3 (hSCP3), the DNA fragments encoding XLR and SCP3 were amplified from A17 tumor cell cDNA; DNA fragments encoding XMR were amplified from mouse testis cDNA; and DNA fragments encoding hSCP3 were amplified from a human testis cDNA library (Clontech) with PCR using a set of primers. The amplified DNAs were subsequently cloned into pMSCV retroviral vector (Clontech). Plasmid constructs were confirmed by DNA sequencing.

Synthetic siRNA specific for Akt was purchased from Dharmacon. XLR and green fluorescent protein (GFP) siRNAs were synthesized using 20-O-ACE-RNA phosphoramides (Dharmacon; see Supplementary Table S1).

**Chemical reagents.** LY294002 (Calbiochem Corp.) for phosphatidylinositol 3-kinase (PI3K) inhibition, API-2 (Calbiochem) for Akt inhibition, SB203580 (Calbiochem) for p38 inhibition, and PD98059 (Stressgen) for extracellular signal-regulated kinase (ERK) inhibition were used for inhibition of the individual kinase pathway.

**Cells.** Six HPV-16 E7–expressing cell lines, TC-1, TC-1 P3 (A17), TC-1/no insert, TC-1/XLR, TC-1/XMR, and TC-1/SCP3, were used as murine tumor models and 293Db/no insert and 293Db/hSCP3 cell lines were used as human tumor models (10). The production and maintenance of TC-1 and TC-1 P3 (A17) cells has been described previously (2). B16, B16F10, EL4, CT26, CMT93, WEHI164, Neuro-2a, and NIH3T3 cell lines were purchased from the American Type Culture Collection. TC-1/no insert, TC-1/XLR, TC-1/XMR, TC-1/SCP3, 293Dp/no insert, and 293Dp/hSCP3 cell lines were generated using the constructed pMSCV encoding no insert, XLR, XMR, SCP3, hSCP3, or XLR by methods described previously (5).

**Flow cytometric analysis.** For in vitro E7-specific CD8+ T-cell activation, TC-1/no insert and TC-1/XLR cells were incubated with an E7-specific CD8+ T-cell line at 0.01:1, 0.1:1, and 1:1 ratio of tumor to T cells for 16 h. Cell surface marker staining of CD8 and intracellular cytokine staining for IFN-γ as well as FACScan analysis were performed using conditions described previously (11–14). To detect MHC class I expression, phycoerythrin (PE)-labeled anti-mouse H-2Kb or H-2Db (BD Biosciences) monoclonal antibody was used. Analysis was done on a Becton Dickinson FACScan with CellQuest software (BD Biosciences). The in vitro CTL assay was performed using methods described previously (5, 11–14) and analyzed by flow cytometric analysis.

**In vivo tumor treatment experiments.** The in vivo tumor treatment experiment using vaccinia virus vaccination or adoptive T-cell transfer using chitosan hydrogel containing 5 μg API-2 (5, 15) was performed as described previously (5).

**Western blot.** A total of 5 × 10^7 cells were used as described previously (5). The primary antibodies phospho-Akt (pAkt; Ser473), Akt, phospho-ERK (pERK; Thr202/Tyr204), ERK, p38 mitogen-activated protein kinase (MAPK), Bcl-w, Bid, Bim, Bad, phospho-Bad (pBad; Ser136), cIAP-1, and XIAP (1:1,000; Cell signaling); Mcl-1, Bcl-2, cIAP-2, Bcl-xL, Bax, HA probe, and survivin (1:1,000; Santa Cruz Biotechnology); Bak and SCP3 (1:1,000; BD Biosciences); E7 (provided by Dr. Ju-hong Jun, Seoul National University, Seoul, Korea); and XLR (provided by Dr. Henri-Jean Garchon, Institut National de la Santé et de la Recherche Médicale, Paris, France) were used for Western blotting followed by the appropriate secondary antibodies. Immunoreactive bands were visualized by enhanced chemiluminescence (ECL; Elpis Biotech) reaction.

**Statistical analysis.** All data are representative of at least two separate experiments. Results for intracellular cytokine staining with flow cytometric analysis and tumor treatment experiments were evaluated by ANOVA. Comparisons between individual data points were made using Student’s t test. All P values <0.05 were considered statistically significant.

**Results**

**Overexpression of XLR in TC-1 P3 (A17) tumor cells leads to an immune resistance to apoptotic cell death induced by E7-specific CD8+ T cells.** In our previous study, we have characterized the immunoresistant A17 tumors and the parental TC-1/P0 tumors and found that A17 tumor cells showed comparable levels of E7 and MHC class I expression and the ability to activate E7-specific CD8+ T cells compared with TC-1 tumors (5). We also sequenced the HPV-16 E7
gene within the A17 tumor cells and found no mutations (data not shown), thus precluding the alteration of the immunodominant epitope of E7. To identify candidate genes that are overexpressed in the immune-resistant TC-1 P3 tumor cell clone A17, we performed a microarray analysis and observed that the X-linked lymphocyte regulated protein pM1 (XLR) showed the highest fold increase in expression in the TC-1 P3 (A17) cells compared with TC-1 P0 cells (Supplementary Table S2). To confirm this, we also performed Western blot analysis to compare the expression of XLR in TC-1 P3 (A17) cells and TC-1 P0 cells. As shown in Fig. 1A, XLR was found to be upregulated in TC-1 P3 (A17) cells compared with TC-1 P0 cells. Furthermore, we have shown that several murine tumor cells express significant levels of XLR (see Supplementary Fig. S1).

Previously, we showed that the immunoresistance of TC-1 P3 (A17) was associated with its resistance against E7-specific CD8+ T-cell–mediated apoptosis, and the observed resistance against apoptosis was found to be associated with the upregulation of antiapoptotic molecules caused by Akt activation (5). Thus, we first compared the apoptotic cell death induced by E7-specific CD8+ T cells in A17 and TC-1 cells. Consistently, we found that A17 tumor cells showed significant resistance to tumor cell killing compared with the TC-1 tumor cells (Fig. 1B). We also found that the percentage of apoptotic cells increased with time (Fig. 1C). We intended to further confirm the link between XLR expression level and resistance to CTL killing by using siRNA technology. For this, A17 cells were transfected with GFP siRNA or XLR siRNA and in turn characterized for expression of XLR by Western blot analysis. XLR expression was completely abolished in XLR siRNA–transfected A17 cells, and this led to reduction in the immune resistance to apoptotic cell death mediated by E7-specific CD8+ T cells compared with GFP siRNA–transfected A17 cells.
cells (Fig. 1D). Thus, our data indicate that overexpression of XLR in TC-1 P3 (A17) cells contributes to immune resistance to apoptotic cell death mediated by E7-specific CD8+ T cells.

**TC-1/XLR tumor cells show comparable levels of E7 expression and the ability to activate E7-specific CD8+ T cells compared with TC-1 tumors but show immune resistance phenotype.** To determine if the expression of XLR in TC-1/P0 cells results in an immune resistance phenotype, we introduced DNA construct encoding XLR into TC-1/P0 cells via a retroviral system. TC-1/P0 cells transduced with no insert (pMSCV) were used as a control (TC-1/no insert). We then performed Western blot analysis to determine the expression of XLR and E7 in TC-1/XLR and TC-1/no insert cells. We observed that TC-1/XLR showed significantly higher expression of XLR compared with TC-1/no insert cells (Fig. 2A). The TC-1/XLR and TC-1/no insert cells also showed comparable expression levels of E7 (Fig. 2A). We further evaluated the phenotypic and functional characteristics of the TC-1/XLR cells. As shown in Fig. 2B, we observed that TC-1/XLR cells showed comparable expression levels of MHC class I compared with the TC-1/no insert cells. Furthermore, to determine the ability of TC-1/XLR cells to

![Figure 2](image_url)

**Figure 2.** Functional characterization of TC-1/XLR tumor cell line. A, Western blot analysis to characterize the expression of XLR and E7 in TC-1/no insert and TC-1/XLR cells. B, flow cytometric analysis to characterize MHC class I expression on TC-1/no insert and TC-1/XLR cells. PE-conjugated anti-mouse H-2D^b^ monoclonal antibody was used to detect MHC class I expression. The isotype antibody was used as the negative control (black profile). C, intracellular cytokine staining and flow cytometric analysis to determine the number of IFN-γ-secreting E7-specific CD8+ T cells induced by TC-1/no insert and TC-1/XLR cells. TC-1/no insert and TC-1/XLR cells were incubated with E7-specific CD8+ T cells at different E:T ratios (0.01:1, 0.1:1, and 1:1) for 16 h. After incubation, cells were stained for CD8 and IFN-γ and analyzed by flow cytometric analysis. D, graphical representation of the tumor volume in mice challenged with TC-1/no insert and TC-1/XLR cells with or without vaccination with Vac-Sig/E7/LAMP-1. Data shown are representative of three independent experiments.
process and present the E7 peptide through the MHC class I pathway, we characterized the ability of TC-1/XLR cells to activate E7-specific CD8+ T cells compared with TC-1/no insert tumors. We observed that TC-1/XLR cells showed a similar ability to activate E7-specific CD8+ T cells in vitro compared with TC-1/no insert tumors (Fig. 2C). This result suggests that the antigen processing and presentation of E7 through the MHC class I was not impaired in TC-1/XLR cells relative to TC-1/P0 cells.

We further determined if the TC-1/XLR tumor cells maintained the immune resistance phenotype similar to the A17 tumors in tumor-bearing mice. As shown in Fig. 2D, TC-1/XLR tumor-challenged mice vaccinated with Vac-Sig/E7/LAMP-1 showed significantly higher tumor volumes over time compared with TC-1/no insert tumors (Fig. 2C). This result suggests that the antigen processing and presentation of E7 through the MHC class I was not impaired in TC-1/XLR cells relative to TC-1/P0 cells.

We further determined if the TC-1/XLR tumor cells maintained the immune resistance phenotype similar to the A17 tumors in tumor-bearing mice. As shown in Fig. 2D, TC-1/XLR tumor-challenged mice vaccinated with Vac-Sig/E7/LAMP-1 showed significantly higher tumor volumes over time compared with TC-1/no insert tumors (Fig. 2C). This result suggests that the antigen processing and presentation of E7 through the MHC class I was not impaired in TC-1/XLR cells relative to TC-1/P0 cells.

We further determined if the TC-1/XLR tumor cells maintained the immune resistance phenotype similar to the A17 tumors in tumor-bearing mice. As shown in Fig. 2D, TC-1/XLR tumor-challenged mice vaccinated with Vac-Sig/E7/LAMP-1 showed significantly higher tumor volumes over time compared with TC-1/no insert tumors (Fig. 2C). This result suggests that the antigen processing and presentation of E7 through the MHC class I was not impaired in TC-1/XLR cells relative to TC-1/P0 cells.

We further determined if the TC-1/XLR tumor cells maintained the immune resistance phenotype similar to the A17 tumors in tumor-bearing mice. As shown in Fig. 2D, TC-1/XLR tumor-challenged mice vaccinated with Vac-Sig/E7/LAMP-1 showed significantly higher tumor volumes over time compared with TC-1/no insert tumors (Fig. 2C). This result suggests that the antigen processing and presentation of E7 through the MHC class I was not impaired in TC-1/XLR cells relative to TC-1/P0 cells.

We further determined if the TC-1/XLR tumor cells maintained the immune resistance phenotype similar to the A17 tumors in tumor-bearing mice. As shown in Fig. 2D, TC-1/XLR tumor-challenged mice vaccinated with Vac-Sig/E7/LAMP-1 showed significantly higher tumor volumes over time compared with TC-1/no insert tumors (Fig. 2C). This result suggests that the antigen processing and presentation of E7 through the MHC class I was not impaired in TC-1/XLR cells relative to TC-1/P0 cells.

We further determined if the TC-1/XLR tumor cells maintained the immune resistance phenotype similar to the A17 tumors in tumor-bearing mice. As shown in Fig. 2D, TC-1/XLR tumor-challenged mice vaccinated with Vac-Sig/E7/LAMP-1 showed significantly higher tumor volumes over time compared with TC-1/no insert tumors (Fig. 2C). This result suggests that the antigen processing and presentation of E7 through the MHC class I was not impaired in TC-1/XLR cells relative to TC-1/P0 cells.

We further determined if the TC-1/XLR tumor cells maintained the immune resistance phenotype similar to the A17 tumors in tumor-bearing mice. As shown in Fig. 2D, TC-1/XLR tumor-challenged mice vaccinated with Vac-Sig/E7/LAMP-1 showed significantly higher tumor volumes over time compared with TC-1/no insert tumors (Fig. 2C). This result suggests that the antigen processing and presentation of E7 through the MHC class I was not impaired in TC-1/XLR cells relative to TC-1/P0 cells.

We further determined if the TC-1/XLR tumor cells maintained the immune resistance phenotype similar to the A17 tumors in tumor-bearing mice. As shown in Fig. 2D, TC-1/XLR tumor-challenged mice vaccinated with Vac-Sig/E7/LAMP-1 showed significantly higher tumor volumes over time compared with TC-1/no insert tumors (Fig. 2C). This result suggests that the antigen processing and presentation of E7 through the MHC class I was not impaired in TC-1/XLR cells relative to TC-1/P0 cells.
tumor cell death of TC-1/XLR cells induced by E7-specific CD8+ T cells.

To determine if the expression of XLR in TC-1 cells results in upregulation of antiapoptotic proteins, we characterized the expression of key proapoptotic and antiapoptotic proteins in TC-1/no insert and TC-1/XLR cells. We found that the expression of antiapoptotic proteins (Bcl-2, Bcl-xL, pBad, survivin, and clAP-2) was significantly increased in the TC-1/XLR cells compared with the TC-1/no insert cells, whereas the expression of key proapoptotic proteins (Bak, Bax, Bim, and Bad) showed no significant difference between TC-1/XLR and TC-1/no insert cells (Supplementary Fig. S4A).

**Treatment of TC-1/XLR tumor cells with the Akt inhibitor API-2 reduces the expression of antiapoptotic proteins, resulting in the abolishment of immune resistance phenotype.** To confirm the role of Akt in the immune resistance of TC-1/XLR apoptotic tumor cell death induced by E7-specific CD8+ T cells, we used a pharmacologic inhibitor of Akt, Akt/protein kinase B signaling inhibitor-2 (API-2), which has been shown to suppress the kinase activity and phosphorylation level of Akt, leading to inhibition of cell growth and induction of apoptosis (16). As shown in Fig. 4A, the expression of the key antiapoptotic proteins (Bcl-xL and Bcl-2) in the TC-1/XLR cells was significantly reduced in the presence of API-2. Consistently, similar phenotypes were observed after siAkt treatment (Supplementary Fig. S4B).

We then characterized the in vivo tumor growth in TC-1/XLR tumor-bearing mice treated with or without the inhibitor. For this, chitosan hydrogel containing 5 μg API-2 was injected intratumorally (5). The chitosan hydrogel was completely degraded and disappeared 7 to 9 days after injection. Thus, the experiment was terminated, and tumor volumes were measured 10 days following T-cell adoptive transfer. As shown in Fig. 4B, tumor-challenged mice treated with adoptive transfer of E7-specific CD8+ T cells in the presence of API-2 showed significantly lower tumor volume compared with tumor-challenged mice treated with E7-specific CD8+ T cells without API-2 treatment. Thus, our data indicate that inhibition of Akt in TC-1/XLR tumor reduces the expression of antiapoptotic proteins, resulting in the abolishment of immune resistance phenotype.

**TC-1/P0 cells transduced with homologous genes from the XLR family lead to activation of Akt, upregulation of antiapoptotic proteins, and induction of the immune resistance phenotype.** To explore whether other genes from the XLR family, which share significant homology with XLR, could induce immune resistance phenotype on TC-1/P0 cells, we aligned the amino acid sequence of XLR gene with other XLR family members, XMR, SCP3, and hSCP3. We observed >60% homology of these genes compared with the XLR gene (see Supplementary Table S3; refs. 17–20). We then characterized the expression and function of SCP3 or XMR in transduced TC-1/P0 cells. As shown in Fig. 5A, TC-1 cells transduced with XMR or SCP3 also showed significant activation of Akt and upregulation of Mcl-1, Bcl-xL, and Bcl-2 compared with the control TC-1/no insert cells.

We also determined if TC-1/SCP3 and TC-1/XMR cells show resistance to the apoptotic cell death induced by E7-specific CTLs. As shown in Fig. 5C, there was a significant reduction of apoptotic cell death in TC-1/XLR, TC-1/SCP3, and TC-1/XMR cells compared with TC-1/no insert cells, whereas there was no significant change in MHC class I expression and ability to activate E7-specific CD8+ T cells in vitro (Fig. 5B).

We further assessed whether these tumor cell lines are resistant to the therapeutic effect of adoptive transfer of E7-specific CD8+ T cells in vivo. As shown in Fig. 5D, TC-1/no insert tumor-challenged mice treated with E7-specific CD8+ T cells showed significantly lower tumor volume over time compared with TC-1/XLR, TC-1/SCP3, or TC-1/XMR tumor-bearing mice treated with E7-specific CD8+ T cells. Taken together, our data suggest that TC-1/P0 cells transduced with homologous genes from the XLR family lead to activation of Akt, upregulation of antiapoptotic proteins, and induction of the immune resistance phenotype.

---

**Figure 4.** Characterization of antitumor effects generated by E7-specific CD8+ T-cell adoptive transfer combined with Akt inhibitor API-2 treatment. A, Western blot analysis to characterize the expression of pAkt, Akt, Bcl-xL, and Bcl-2 in DMSO or Akt inhibitor API-2–treated TC-1/XLR cells. B, bar graph representing tumor volumes from tumor-challenged mice treated with or without API-2 in the presence or absence of E7-specific CD8+ T cells on 10 d after tumor challenge. Tumor volumes from TC-1/XLR tumor were recorded twice per week for 10 d following adoptive transfer.
Human 293Db cells transduced with hSCP3 are highly resistant to apoptotic cell death induced by CTL killing. Our data indicate that SCP3 plays an important role in the immune resistance phenotype in murine tumor cells. Thus, we characterized the expression of the hSCP3 gene in cervical tissue from various stages of human cervical cancer by immunohistochemical staining analysis using antibody against hSCP3. hSCP3 expression was observed in human cervical cancer cells but not in the surrounding stromal tissue (Supplementary Fig. S5A). In general, the higher stages of cervical cancer showed higher level of hSCP3 expression compared with lower-stage cervical cancers or normal cervical tissue (Supplementary Fig. S5B). Thus, our data indicate the upregulation of hSCP3 in human cervical cancers.

To determine if human tumor cells ectopically expressing SCP3 were resistant to CTL-induced apoptosis, we characterized the function of human 293Db cells retrovirally transduced with hSCP3. The expression of hSCP3 in the transduced 293Db cells is shown in Fig. 6A. As shown in Fig. 6B, 293Db/hSCP3 cells showed significant expression of pAkt compared with the control 293Db/no insert cells. Furthermore, 293Db/hSCP3 cells showed a significant upregulation in key antiapoptotic proteins such as Bcl-xL and Bcl-2 compared with the control (Fig. 6B). We also determined if the immune resistance of 293Db/hSCP3 cells is related to the apoptotic cell death induced by CTL killing. As shown in Fig. 6C and D, there was a significant reduction of apoptotic cell death in 293Db/hSCP3 cells compared with 293Db/no insert cells. Thus, our data indicate that human tumor cells transduced with hSCP3 are highly resistant to apoptotic cell death induced by cytotoxic T cells.

**Discussion**

In the current study, we identified XLR as a candidate molecule that is overexpressed in immune-resistant TC-1 P3

![Figure 5](https://example.com/figure5.png)

**Figure 5.** Characterization of expression of XLR family genes in the TC-1/no insert and TC-1/XLR tumor cells. A, Western blot analysis to characterize the expression of SCP3 in TC-1/no insert and TC-1/SCP3 and HA expression in the TC-1/no insert and TC-1/XMR/HA cells and pAkt, total Akt, Mcl-1, Bcl-xL, and Bcl-2 expression in TC-1 cells expressing the XLR homologues. B, flow cytometric analysis to characterize MHC class I expression on TC-1 cells expressing the XLR homologues compared with TC-1/no insert cells and in vitro T-cell activation to determine the number of IFN-γ-secreting E7-specific CD8+ T cells induced by TC-1 cells expressing the XLR homologues compared with TC-1/no insert cells. MFI, mean fluorescence intensity. C, bar graph depicting percentage of apoptotic TC-1 cells expressing the XLR homologues compared with TC-1/no insert cells incubated with E7-specific CD8+ T cells at 1:1 ratio for 4 h. D, graphical representation of the tumor volume in mice challenged with TC-1/XLR, TC-1/SCP3, or TC-1/XMR tumor cells with or without adoptive transfer of E7-specific CD8+ T cells. Data shown are representative of three independent experiments.
We found that the expression of XLR in TC-1/P0 cells could induce an immune resistance phenotype. Thus, we have identified a novel immune resistance mechanism through characterization of the overexpressed protein in immune-resistant tumors by microarray analysis. We report that XLR and its homologues render tumor cells resistant to apoptosis induced by CTL killing possibly through the activation of Akt, leading to the upregulation of antiapoptotic proteins, thus resulting in the immune-resistant phenotype. The Akt-dependent resistance to CTL killing was also observed in other tumor cell line models, including EL4 and LLC cell lines (data not shown). This is consistent with our previous study wherein we showed that activation of PI3K/Akt pathway represents a new mechanism of immune escape (5). XLR is a transcriptional regulatory protein. It is conceivable that overexpression of XLR in tumor cells may result in modulation of proteins important for the Akt activation pathway, such as PI3K and PI3K-dependent kinase 1 (see refs. 21, 22 for reviews). However, there was no difference observed in expression level of phosphatase and tensin homologue (PTEN), which has been known as a central negative regulator of PI3K/Akt signal transduction cascade, in TC-1/XLR and TC-1/no insert cells. Interestingly, we observed that rapamycin treatment led to reverse the immune resistance to CTL killing in TC-1/XLR cells, which had increase of phosphorylated mammalian target of rapamycin (mTOR) compared with TC-1/no insert.8 These data suggest that mTOR could be one of downstream signaling pathways that are activated by XLR-mediated Akt activation. Thus, it will be interesting to explore that the mTOR could form a positive feedback loop of Akt signaling provoked by ectopic expression of XLR. However, we do not exclude other possibility that XLR and its homologue may interact with and, thus, modulate molecules that are associated with Akt signaling.

In our study, we observed that several cervical cancers show significant expression of the XLR homologue, hSCP3. The murine XLR gene does not have a human counterpart, but the murine SCP3 gene belongs to the XLR family and has 62% homology with XLR (see Supplementary Table S3; ref. 18). hSCP3 shares 63% homology with XLR. We also showed that human tumor cells transduced with hSCP3 could lead to activation of Akt, upregulation of antiapoptotic proteins, and induction of the immune resistance phenotype.

8 Unpublished data.
Thus, it will be important to further determine if overexpression of hSCP3 is a common phenomenon in advanced-stage cancers. This could potentially serve as an important mechanism for tumor immune evasion.

In conclusion, this is the first report, to our knowledge, to show that XLR and its human homologue, typical cancer testis antigens, are expressed in immune-resistant mouse tumor cells and human cervical cancer cells, respectively, and play a role in the resistance of tumor cells to CTL killing. On the basis of these findings, further expression profile studies are planned in clinical specimens from patients with HPV-16-associated high-grade cervical intraepithelial neoplasia lesions who were vaccinated with an HPV-16 E7 therapeutic DNA vaccine but failed to show lesion regression. Our study has important implications for future development of a novel strategy for enhancing cancer immunotherapy against immune-resistant tumor cells.

References


Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

National Cancer Institute Specialized Program of Research Excellence grants P50 CA909252, P50 CA96784-06, and R01 CA114425-01; National Research Foundation grant R11-2005-017–0003-0 funded by Ministry of Education, Science and Technology; Korea Science and Engineering Foundation grant 2009-0079732 funded by Ministry of Education, Science and Technology; National R&D Program for Cancer Control grant 070355S; and Korea Healthcare Technology R&D Project grant A062260, Ministry of Health and Welfare, Republic of Korea.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received 11/04/2009; revised 02/12/2010; accepted 02/16/2010.

3070 Cancer Res; 70(8) April 15, 2010 Cancer Research

Downloaded from cancerres.aacrjournals.org on July 22, 2017. © 2010 American Association for Cancer Research.
Ectopic Expression of X-Linked Lymphocyte-Regulated Protein pM1 Renders Tumor Cells Resistant to Antitumor Immunity

Tae Heung Kang, Kyung Hee Noh, Jin Hee Kim, et al.

Cancer Res 2010;70:3062-3070.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/70/8/3062

Supplementary Material
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2010/04/15/70.8.3062.DC1

Cited articles
This article cites 22 articles, 12 of which you can access for free at:
http://cancerres.aacrjournals.org/content/70/8/3062.full#ref-list-1

Citing articles
This article has been cited by 3 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/70/8/3062.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.