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Original Article

Spontaneous metastases in immunocompetent mice harboring a primary tumor driven by oncogene latent membrane protein 1 from Epstein–Barr virus

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ABSTRACT

Background: In vitro and clinical studies suggest that the oncogene LMP1 (latent membrane protein 1) encoded by Epstein–Barr virus (EBV) plays a role in the development of nasopharyngeal carcinoma (NPC) and the formation of metastases in immunocompetent individuals. However, whether LMP1 itself is sufficient to drive these events in immunocompetent hosts remains elusive due to the lack of appropriate experimental models. The aim of this study was to study LMP1-dependent tumorigenesis and metastasis in BALB/c mice inoculated with BALB/c-3T3 cells expressing N-LMP1 (a Taiwanese NPC variant).

Methods: Following cancer cell inoculation, metastasis formation was monitored over time using PCR analysis of LMP1 as tumor marker. We also used a luciferase (Luc)-containing N-LMP1 and bioluminescent imaging (BLI) to monitor metastasis formation in a non-invasive manner.

Results: N-LMP1 appeared early in draining lymph nodes and in various distant organs before the rapid growth of the primary tumor. Lung metastasis was observed by BLI and

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further confirmed by histological examination. Furthermore, we detected luciferase signals in the lungs, even before the animals were sacrificed.
Conclusions: Our results demonstrate the high metastatic character of N-LMP1 in immunocompetent hosts. Systemic tumor dissemination occurs even before aggressive tumor growth at the primary site, suggesting that early treatment of primary LMP1-associated tumors and distant micro-metastases is critical to achieve positive results.

At a glance commentary

Scientific background of the subject

Epstein–Barr virus (EBV)-encoded oncogene latent membrane protein 1 (LMP1) has been shown to play a role in the metastasis of nasopharyngeal carcinoma (NPC), an EBV-associated cancer that uniquely develops in immunocompetent individuals. We sought to examine whether and when LMP1 drives the formation of spontaneous metastasis in the immune intact host using a mouse tumor model established by N-LMP1, a LMP1 variant isolated from Taiwanese NPC patients.

What this study adds to the field

The primary tumor initiated by N-LMP1 showed high metastatic potential in the immunocompetent host. Systemic tumor dissemination occurred immediately after angiogenic switch but before aggressive tumor growth at the primary site. This finding suggests a critical time window for LMP1-based therapeutic interventions that could be used to concomitantly treat primary tumor and distant metastases.

Cancer is a leading cause of death worldwide and survival rates rapidly decline once metastases occur [1]. Metastasis development is a multi-step process characterized by the invasion-metastasis cascade [2–4]. This process involves cancer cell invasion into surrounding tissues, intravasation into nearby blood vessels, passage into the circulation, followed by homing into distant tissues, formation of new micrometastasis foci which eventually grow to form macroscopic secondary tumors. Tumor cell lines have been used to study the growth and motility of cancer cells following their inoculation into the tail vein of mice [5–7]. The role of the microenvironment is becoming more and more important in the study of factors regulating metastasis formation [8]. Unfortunately, *in vitro* cell culture does not reflect the importance of the microenvironment, nor does intravenous tail vein administration simulate the natural course of spontaneous metastasis. Therefore, establishing a spontaneous metastasis mouse model is of paramount importance in the study of tumor metastasis.

Bioluminescent imaging (BLI) has been recently developed to facilitate *in vivo* monitoring of cellular processes in various pathophysiological conditions [9–11]. BLI is a non-invasive imaging technique involving the generation of cold light by luciferase-expressing cells in animals following administration of luciferin substrate. Cold-light signals are then imaged using an external detector, allowing dynamic visualization of tumor-associated properties in living animals.

Nasopharyngeal carcinoma (NPC) is a malignant retronasal tumor that is endemic in Southern Asia and Taiwan [12]. Expression of the oncogene LMP1 (latent membrane protein 1) encoded by Epstein–Barr virus (EBV) has been associated with NPC pathogenesis [13]. NPC arises in immunocompetent hosts despite active humoral and cellular responses against EBV antigens at the systemic level [14]. Metastasis of NPC to regional lymph nodes and distant organs has been documented [15]. In clinical studies, LMP1 has been detected in local lymph nodes and distant tissues containing NPC metastasis by using immunohistochemistry. The cumulative metastasis rate has been estimated at 66.8% (269/403) in cases associated with LMP1 expression and 47.0% (148/315) in those where LMP1 expression is absent [16]. However, other studies have failed to demonstrate a link between LMP1 expression and the degree of tumor metastasis [17–19]. To date, metastasis development remains a problem in NPC treatment, and there are currently few animal models available to study spontaneous metastases in immunocompetent mice.

The cancer cell transformation role of LMP1 was initially described in a B-lymphoma, called B-LMP1 [20]. Later, LMP1 variants with a 30-nucleotide deletion at the 3′-end of the gene along with point mutations within the gene were observed in Chinese NPC [21,22]. The variant isolated from NPC patients in Taiwan, designated N-LMP1, shows the 30-nucleotide deletion but mutations at different regions than the ones identified previously [23]. In contrast to the prototype B-LMP1, N-LMP1-transfected BALB/c-3T3 cells (3T3/N-LMP1) exert a more potent oncogenic activity and form solid tumors in nude mice [24]. Studies have shown that N-LMP1 downregulates the expression of cell adhesion molecules; upregulates the activity of matrix metalloproteinases 1 and 9; and increases cell motility [25,26]. Furthermore, the invasive and metastatic potential of NPC cells is induced by N-LMP1 expression, and is inhibited when LMP1 expression is abrogated using small hairpin RNA *in vitro* and in immunodeficient mice [27,28]. However, whether tumor cells expressing N-LMP1 alone may metastasize in immunocompetent individuals has not been examined so far.

One characteristic of NPC is the massive leukocyte infiltration observed in tumor tissues [29], suggesting a role for components of the immune system in tumor pathogenesis. To study the contribution of immune cells in N-LMP1-driven oncogenesis, we previously established an N-LMP1-derived tumor model in syngeneic immunocompetent mice using tumorigenic 3T3/N-LMP1 cells [30]. Using this model, we report here the possibility to track tumor metastasis by using PCR analysis of N-LMP1 as tumor marker. Furthermore, we show that BLI can be used to follow the occurrence of spontaneous N-LMP1-derived tumor metastasis in immunocompetent hosts.