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Evolution of animal models in cancer vaccine development

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ABSTRACT

Advances in cancer vaccine development are facilitated by animal models reflecting key features of human cancer and its interface with host immunity. Several series of transplantable preneoplastic and neoplastic mouse mammary lesions have been used to delineate mechanisms of anti-tumor immunity. Mimicking immune tolerance to tumor-associated antigens (TAA) such as HER2/neu, transgenic mice developing spontaneous mammary tumors are strong model systems for pre-clinical vaccine testing. In these models, HER2 DNA vaccines are easily administered, well-tolerated, and induce both humoral and cellular immunity. Although engineered mouse strains have advanced cancer immunotherapy, basic shortcomings remain. For example, multiple mouse strains have to be tested to recapitulate genetic regulation of immune tolerance in humans. Outbred domestic felines more closely parallel humans in the natural development of HER2 positive breast cancer and their varying genetic background. Electrovaccination with heterologous HER2 DNA induces robust adaptive immune responses in cats. Importantly, homologous feline HER2 DNA with a single amino acid substitution elicits unique antibodies to feline mammary tumor cells, unlocking a new vaccine principle. As an alternative approach to targeted vaccination, non-surgical tumor ablation such as cryoablation induces anti-tumor immunity via *in situ* immunization, particularly when combined with toll-like receptor (TLR) agonist. As strategies for vaccination advance, non-invasive monitoring of host response becomes imperative. As an example, magnetic resonance imaging (MRI) and positron emission tomography (PET) scanning following administration of tryptophan metabolism tracer [11C]-alpha-methyl-tryptophan (AMT) provides non-invasive imaging of both tumor growth and metabolic activities. Because AMT is a substrate of indoleamine-pyrrole 2,3-dioxygenase (IDO), an enzyme that produces the immune regulatory molecule kynurenine, AMT imaging can provide novel insight of host response. In conclusion, new feline models improve the predictive power of cancer immunotherapy and real-time PET imaging enables mechanistic monitoring of host immunity. Strategic utilization of these new tools will expedite cancer vaccine development.

1. Introduction

To predict human response to cancer vaccines, candidate regimens should be interrogated in a fully functional immune system. To date, only intact animals carrying all immunological constituents can mimic human responses to vaccination or associated immune modulation. Therefore, the choice of animal models is critical in cancer vaccine research. Comparative oncology models where animal malignancies express analogous tumor-associated antigens (TAA) and exhibit disease progression similar to humans may provide greater predictive power of treatment outcomes. Artificial or foreign antigens frequently used in vaccine studies may induce striking reactivity in pre-clinical models (*e.g.* ovalbumin), but the findings often fall short in predicting patient response. Even genetically modified inbred mice with spontaneous tumor development may not fully recapitulate human tumor immunology. These pre-clinical model inadequacies may have hindered clinical translation of cancer vaccines. At a time when cancer immunotherapy is coming to the forefront, it is opportune to re-examine the usage of animal models. Rather than conducting a comprehensive review of all models ever tested, we will discuss animal models and mammary tumor systems with a focus on HER2/neu antigen to show the utility, futility and new development of animal models.

2. Early animal models and tumor cell lines

Early studies of mammary tumors in syngeneic mice captured many biological parallels between human breast cancer and spontaneous mouse mammary tumors. The cell lines derived from these tumors continue to be strong tools in cancer immunotherapy research. In the 1960s and 1970s, mouse mammary tumor virus (MMTV) [1,2], hormones and chemical carcinogens [3–5] were

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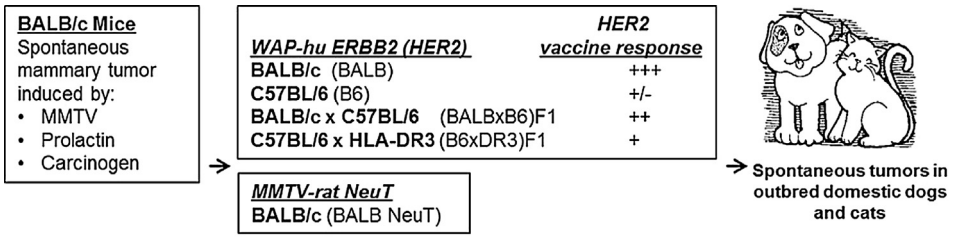

Fig. 1. Evolution of pre-clinical models for cancer vaccine research. Initial breast cancer models began in mice using various transforming agents to induce spontaneous mammary tumors. More advanced mouse models in different strains were created to better recapitulate self-tolerance observed in humans. Outbred dogs and cats can serve as a natural model for tumorigenesis with immunity more representative of humans relative to syngeneic mice.

used as experimental tumor inducing agents (Fig. 1, left panel). MMTV induces mammary tumors by insertional activation of oncogenes or genes critical in tumorigenesis [6,7]. A number of MMTV activated genes are also deregulated and/or mutated in primary human breast cancer, suggesting parallel biology between breast cancer and MMTV induced tumors [6,8]. Infectious MMTV particles can be introduced into any mouse strain through virally contaminated milk [9]. For example, BALB/cfC3H (BfC3H) mice were derived from BALB/c pups fostered by MMTV infected C3H mothers. The pups were infected via nursing and ultimately went on to develop mammary tumors. In MMTV induced primary tumors or tumor cell lines, MMTV associated antigens are often down-regulated to avoid immune recognition, but when expression is exogenously induced they become potent foreign antigens in wild type mice.

From a single MMTV induced mouse mammary tumor in a BfC3H mouse, a panel of tumor cell lines were derived and characterized by our group [10,11]. MMT lines 66, 67, 68H, 168 and 4.10 were in the initial panel. Of these lines, only 68H expressed significant levels of MMTV envelop protein antigens, although such expression can be induced in the other lines [12]. Through *in vivo* selection for metastatic behavior and *in vitro* selection for ouabane and/or thioguanine resistance, two highly metastatic lines 4T07 and 4T1 were established from 4.10. Selection for ouabane or thioguanine resistance was primarily for research convenience (*e.g.* enumeration of tumor cells in micro foci by culturing dissociated tumor cells in test drugs). The thioguanine resistant line 4T1 has been used in numerous tumor metastasis studies [13]. 4T1 tumors produce large quantities of myeloid cell stimulating cytokines, which cause tumor-bearing mice to develop splenomegaly with heavy myeloid cell infiltration. Many studies of myeloid derived suppressor cells (MDSC) are conducted with the 4T1 model, where myeloid cell expansion may be exaggerated. Still, 4T1 and its sister MMT lines are powerful tools in cancer research, especially when experimental results are interpreted with consideration of their derivation history [11].

Another series of mammary tumor cell lines were derived from tumors that arose in serially passaged, preneoplastic mammary hyperplastic alveolar nodules (HAN) that developed in a BALB/c female mouse with uninhibited prolactin secretion [3,4]. HAN tissue can be serially passaged in cleared (*i.e.* surgical removal of host mammary gland) mammary fat pads of 3 week old females, with this tissue maintaining the hyperplastic alveolar morphology for several months until spontaneous tumors arise from it. From these spontaneous tumors, MMT lines D2F2 and D2A1 were established [14]. Although these lines are not infected with milk-transmitted MMTV, nor selected with drugs, spectral karyotyping analysis showed a number of chromosomal aberrations, suggesting genetic alterations as a result of tumorigenesis and cell line derivation [15]. The D2F2 line and its derivatives transfected to express oncogenes or TAAs are frequently used in our and others' studies [11,16–21].

3. Rat neu transgenic (Tg) mice

When genetic engineering of mice became possible, mice expressing human TAA were generated to enable the study of immune reactivity or tolerance to TAA. Tg mice expressing rat neu oncogene under the MMTV promoter have been generated in several genetic backgrounds [22]. Among them, BALB NeuT (NeuT) mice are BALB/c mice expressing a transforming rat neu gene (Fig. 1, middle, bottom panel) [23,24]. These mice are maintained as heterozygotes by breeding with BALB/c mice, as homozygotes are not viable. All NeuT females develop up to 10 spontaneous mammary tumors, one from each mammary gland, around the age of 17–19 weeks. NeuT mice show immune tolerance and reduced response to rat neu immunization as reported by us and others [18,25–29]. Several neu positive mammary tumor lines have been established from NeuT spontaneous tumors such as TUBO [30], Bam1a and BamIR5 [31], which grow progressively in both NeuT and wild type BALB/c mice. TUBO and Bam1a tumors are highly dependent on the HER2/neu signaling pathway for their survival and are very sensitive to the cytotoxic effect of tyrosine kinase inhibitors or anti-neu antibodies [18,31,32]. BamIR5 cells were selected in culture to be gefitinib resistant. In contrast to TUBO or BAM1a cells, D2F2/neu cells transfected to express rat neu are resistant to anti-neu antibody, but sensitive to T-cell cytotoxicity. D2F2/neu represents an antibody resistant HER2/neu positive tumor cell line [15]. Together, wild type BALB/c and NeuT mice, with tumor lines TUBO, BAM1a, BAM IR5, D2F2 and D2F2/neu constitute a comprehensive test system to investigate neu related immune responses, neu targeted vaccine efficacy and other immunotherapy approaches.

Another strain of rat neu Tg mice, FVB/N-Tg(MMTVneu) 202Mul/J (FVB/N), express wild type neu under the MMTV promoter/enhancer [33]. Approximately half of the females develop spontaneous mammary tumors around the age of 28 weeks [33]. FVB/N mice have also been used extensively in HER2/neu vaccination studies that target rat neu [34–36]. However, the most relevant animal model for studying immune tolerance to rat neu remains the wild type rat, which exhibits natural tolerance to rat neu. Disis et al. showed the induction of T-cell immunity against rat neu in rats using heterologous human HER2 protein [37] or rat neu peptides [38]. Following this concept, a number of human clinical trials were conducted with HER2 peptides [39,40] (http://clinicaltrials.gov). Reactivity to HER2 peptides was found in our human HER2 DNA vaccinated breast cancer patients [41], which supports the feasibility of eliciting immune reactivity to self HER2.

4. Human HER2 Tg mice

Using the whey acidic protein (WAP) promoter, which is expressed in mammary epithelial cells of pregnant or lactating mice, we generated HER2 Tg mice in both C57BL/6 (B6) (B6.Cg-Tg(Wap-ERBB2)229Wzw/J, Jackson laboratory) and BALB/c (BALB) backgrounds [42,43]. Immune tolerant status to human HER2 was

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