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EDITORIAL

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Mammary carcinoma: toward a realistic mouse model of incurable cancers

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ABSTRACT

As long as breast cancer (BC) stays under immunosurveillance, it can be controlled by treatments eliciting anticancer immune responses. However, once BC escapes immunosurveillance, it becomes therapeutically uncontrollable. A paper in the *Journal for ImmunoTherapy of Cancer* describes a new hormone receptor-positive BC cell line generating incurable tumors in C57BL/6 mice.

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Main text

The complex relationship between cancer and the immune system is governed by the 3E rule: elimination, equilibrium and escape[1]. Most potential cancers are eliminated before they become detectable, usually at the microscopic stage. Some cancers develop into smoldering lesions in which malignant cells and immune effectors engage in a combat that keeps the tumor at an early, localized stage. In this precarious equilibrium phase, the (re)activation of the anticancer immune response by therapeutic agents has a high chance of yielding long-term effects. However, certain cancers manage to fully escape from immunosurveillance, hence invading local tissues and disseminating to distant sites uncontrollably. The 3E rule was first documented in a mouse model of fibrosarcoma induced by 3-methylcholanthrene (MCA). MCA-induced tumors developed with lower incidence and longer latency in immunocompetent mice compared to *Rag2*$^{-/-}$ immunodeficient mice (lacking *Rag2* recombinase and deprived of functional lymphocytes)[2]. Cell lines derived from MCA-induced tumors in *Rag2*$^{-/-}$ mice could always be transplanted into immunodeficient counterparts; however, when inoculated in immunocompetent mice, they regressed and failed to establish tumors in some hosts[2]. In contrast, cells generated in immunocompetent mice could always be transplanted both into immunocompetent and immunodeficient recipients[2]. This observation illustrates the phenomenon of tumor 'immunoediting' (Figure 1a). To develop cancer in the context of a complete immune system, cancer must break through natural immunosurveillance, for instance by losing immunogenic properties (immunoevasion) and/or by acquiring the capacity of actively subverting the immune response (immunosuppression)[3].

We validated the 3E concept in a well-characterized mouse model of hormone receptor-positive (HR$^+$) breast cancer (BC), which is the most frequent subtype affecting women, using the combination of the progesterone analog medroxyprogesterone acetate (MPA) with the DNA damaging agent 7,12-dimethylbenz[a]anthracene (DMBA) to generate tumors[4,5]. MPA/DMBA (M/D)-induced mammary carcinomas develop much more quickly in T and NK cell-immunodeficient mice (lacking also the gamma chain of interleukin-2 receptor; genotype: *Rag2*$^{-/-}$*Il2rg*$^{-/-}$) than in immunocompetent C57BL/6 controls[4] (Figure 1a). Moreover, M/D-derived BC cells (named MGT cells) recovered from *Rag2*$^{-/-}$ mice always failed to proliferate when they were transplanted into immunocompetent mice at low dose (5×10^5 cells), contrasting with the fact that cells recovered from immunocompetent mice always grew on *Rag2*$^{-/-}$ recipients[4]. However, only in a small portion of cases (in 2 out of 84 mice, ~2%), BC cells from immunocompetent females gave rise to palpable tumors when inoculated in syngeneic immunocompetent recipients[4], suggesting anticancer control of these tumors by T and/or NK cell-mediated immunosurveillance and/or cancer cell rejection due to their immunogenic properties (Figure 1a).

Yet, the difficulty of transplanting syngeneic M/D-derived BC cell lines in immunocompetent mice impedes studying HR$^+$BC in its most aggressive stage, when it evades immunosurveillance. To overcome this, we modified the culture strategy to generate M/D-derived BC cells and injected ten times more cells (5×10^6 cells) orthotopically in immunocompetent C57BL/6 mice. Such modifications could help to overcome the immune barrier by favoring the culture retention of cells evading immunoselection and/or by causing a 'mass effect' (that might be explained by cancer cell-mediated immunosuppression)[1]. We generated eight M/D-derived cell lines (named BXBC cells) that allow the development of tumors in all transplanted mice (in 30 out of 30

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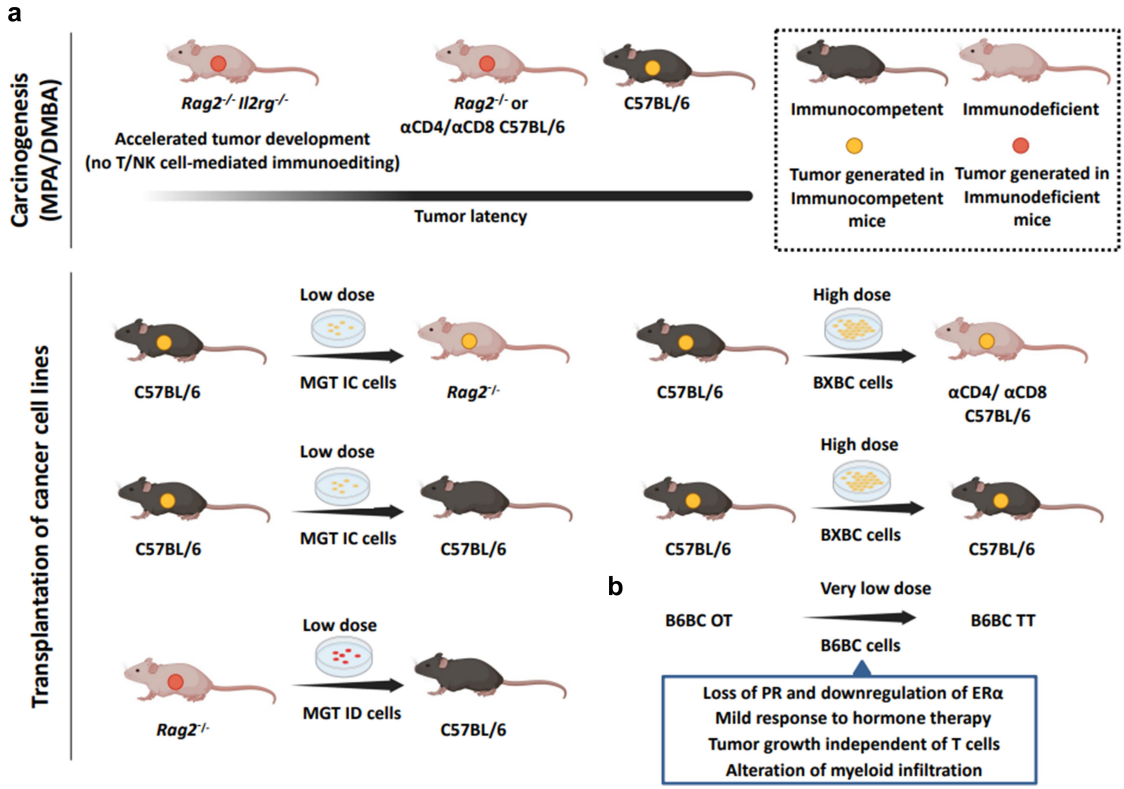

Figure 1. Schematic representation of the principles of cancer immunosurveillance and the generation of B6BC transplantable tumor (TT). (a) Principles of immunosurveillance in the context of hormone receptor-positive (HR+) breast cancer (BC). BCs induced by the progesterone analogue medroxyprogesterone acetate (MPA) combined with the DNA damaging agent 7,12-dimethylbenz[a]anthracene (DMBA) develop with a delay in immunocompetent C57BL/6 mice as compared to severe immunodeficient mice lacking T and NK cells (phenotype *Rag2*$^{-/-}$ *Il2rg*$^{-/-}$), reflecting T and NK cell-mediated immunoediting. Of note, animals lacking functional T cells (phenotype *Rag2*$^{-/-}$ or depleted of CD4$^+$ and CD8$^+$ cells) show a similar tumor latency to C57BL/6 mice. Transplantation of tumor-derived cell lines from immunocompetent to immunodeficient is possible but not vice versa. Moreover, the dose (high = 5 × 10^6 cells or low = 5 × 10^5 cells) and the way of generation of the cancer cells that are transferred (MGT cells or BXBC cells among which B6BC cells), influence transplantability of tumors among immunocompetent mice. (b) Molecular differences between original (OT) and transplanted (TT) B6BC cancers generated by the injection of very low doses of B6BC cells (2 × 10^5 cells), as determined by histopathological, immunological and single nucleus RNA sequencing analyses. Figure created with Biorender.com.

mice, 100%)[6] (Figure 1a), in some cases even at very low doses (2 × 10^5 cells) (Figure 1b).

Since there was no HR$^+$BC cell line transplantable from C57BL/6 mice, the most-studied inbred mouse strain, among the eight cell lines, we selected the one called B6BC that expressed functional estrogen (ER) and progesterone receptors (PR)[6]. We performed exhaustive histopathological, immunological, and single nucleus RNA sequencing (snRNAseq) analyses of the original M/D tumor (OT) giving rise to B6BC cells and of the transplanted tumors (TT) derived from them (Figure 1b) to understand their evolution (from OT to TT) and to compare them with other commonly used BC mouse cell lines.

As compared to B6BC OT, all malignant cells contained B6BC TT exhibited shifts in their transcriptome that may be interpreted as signs of epithelial-mesenchymal transition[6]. However, B6BC OT cancer cells separated in two distinct cell populations (one of which was shared with B6BC TT) in snRNAseq analyses, indicating a greater cancer cell heterogeneity in B6BC OT. Despite deriving from an ERα$^+$ PR$^+$ M/D tumor and ERα$^+$ PR$^+$ B6BC cells, B6BC TT expressed ERα but lost PR upon *in vivo* inoculation and partially responded to ER-targeted agents like tamoxifen and fulvestrant, without showing any complete pathological responses[6] (Figure 1b). This resembles the partial and transient responses previously observed on prophylactic oral tamoxifen treatment in M/D-induced tumors[4]. Of note, this treatment failed to control tumor growth in *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice[4].

Compared to the healthy mammary gland, M/D-induced BCs were less infiltrated by CD4$^+$ and CD8$^+$ T cells[4]. However, the depletion of these cells did not impact tumor development, except if accompanied by NK cell ablation[4] (Figure 1a). Similarly, B6BC TT showed a scarce infiltration by T lymphocytes (and NK cells) and were not affected by CD4$^+$/CD8$^+$ depletion before or after B6BC implantation[6]. Accordingly, PD-1 blockade was unable to slow B6BC TT progression. Although B6BC TT mildly responded to the immunogenic chemotherapeutic mitoxantrone, CD4$^+$/CD8$^+$ depletion did not condition the therapeutic outcome[6], suggesting an incapability of these tumors to elicit T cell responses (Figure 1b).

The most abundant immune population in B6BC tumors consisted of CD11b$^+$ myeloid cells. Blockade of CD11b mildly reduced the B6BC TT growth but had no impact on M/D-induced tumors or other cell line TT models[6] (Figure 1b). In B6BC TT, CD11b neutralization drove the emergence of otherwise low-abundant macrophages, some of which are present in the healthy mammary tissue[7]. Moreover, snRNAseq analyses revealed the expansion of one myeloid population in B6BC TT (absent in B6BC OT) expressing *Spp1*, a marker linked to pro-angiogenic (BC) tumor-associated macrophages[8].

Importantly, B6BC cells were inoculated at very low doses (2×10^5 cells)[6], indicating that beyond cell numbers, the functional properties of transplanted cells are essential to overcome immunosurveillance and facilitate tumor development.

Altogether, it appears that B6BC TT resembles advanced ERα$^+$BC, which is independent of CD4$^+$ and CD8$^+$ immunosurveillance, but rather configures a tumor-supportive myeloid immune microenvironment. Of note, B6BC TT responded in a partial, never complete, manner to hormonotherapy and chemotherapy (with anthracyclines), which require T-cell dependent immune responses to be fully efficient[4,9,10]. Moreover, B6BC TT completely failed to respond to immunotherapy blocking PD-1[6]. In this sense, B6BC TT pose a true challenge reminiscent of immune-escaped (currently) intractable advanced human BC. In this context, it should be noted that B6BC cells expressed low levels of ERBB2 and failed to respond to the ERBB2 inhibitor lapatinib *in vitro*, as compared to ERBB2$^+$ human BC cells[6]. However, B6BC cells reduced their proliferation upon treatment with the CDK4/6 inhibitors abemaciclib, palbociclib, and ribociclib *in vitro*[6]. It remains to be determined whether B6BC TT also respond to such treatments *in vivo*. A current preclinical limitation of B6BC TT is that so far these tumors have only been evaluated in the context of unresectable advanced disease treated with systemic therapies without exhibiting signs of hepatic or pulmonary macrometastasis[6]. Thus, it will be interesting to determine whether B6BC TT could constitute a representative preclinical model of other relevant disease settings such as local or metastatic disease.

Disclosure statement

GK has been holding research contracts with Daiichi Sankyo, Eleor, Kaleido, Lytix Pharma, PharmaMar, Osasuna Therapeutics, Samsara Therapeutics, Sanofi, Tollys, and Vascage. GK is on the Board of Directors of the Bristol Myers Squibb Foundation France. GK is a scientific co-founder of EverImmune, Osasuna Therapeutics, Samsara Therapeutics, and Therafast Bio. GK is on the scientific advisory boards of Hevolution, Institut Servier, and Longevity Vision Funds. GK is the inventor of patents covering therapeutic targeting of aging, cancer, cystic fibrosis, and metabolic disorders. GK's wife, Laurence Zitvogel, has held research contracts with Glaxo Smyth Kline, Incyte, Lytix, Kaleido, Innovate Pharma, Daiichi Sankyo, Pilege, Merus, Transgene, 9m, Tusk, and Roche, was on the Board of Directors of Transgene, is a cofounder of everImmune, and holds patents covering the treatment of cancer and the therapeutic manipulation of the microbiota. GK's brother, Romano Kroemer, was an employee of Sanofi and now consults for Boehringer-Ingelheim. The funders had no role in the design of the study; in the writing of the manuscript, or in the decision to publish the results.

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