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Editorial

Rethinking immunotherapy for brain cancers in the light of cancer complexity

Despite aggressive treatments including surgical resection, radiation therapy, and cytotoxic chemotherapy, brain cancer remains incurable with a median survival under 15 months and a 2-year survival of 26.5 per cent[1,2]. The failure of conventional oncology to eradicate glioblastoma, the most common malignant primary brain tumour, has prompted investigators to look for new and more targeted therapeutic options as well as for improved prognostic biomarkers[3]. It is recognized that brain cancer emerges from multiple alterations that induce changes in expression patterns of genes and proteins that function in complex networks controlling critical cellular functions[4]. A primary task of the tumour research is the translation of molecular biomarkers into clinical practice. However, there is still not agreement with regard to the sequence and nature of steps that need to be taken to warrant efficient translation of prognostic and/or predictive biomarkers into clinical use and to the introduction of novel therapeutic strategies[5].

It was once thought that the nervous system (NS) was an immune privileged organ[3]. The central NS features in support of this theory included the blood-brain barrier, the lack of lymphatic vessels and lymph nodes and the low numbers of circulating T-lymphocytes in the NS. Further, there is less human leucocyte antigen (HLA) presentation and absence of antigen presenting cells (APCs) in the NS when compared with other organs. Under physiologic conditions, the brain hosts several immune cell populations[6]. The recent success of immunotherapy in the treatment of various cancers has renewed interest in vaccine therapy for the treatment of malignant brain tumours[5,7,8]. A prerequisite for successful immunotherapy is the identification of tumour-associated antigens (TAA) that can be recognized by T-lymphocytes. Each T-lymphocyte expresses a unique T-cell antigen receptor that confers specificity for a particular peptide sequence of the target antigen. Cancer-testis antigens (CTA) have been proposed as a suitable family of candidate TAA[7,9]. Their immunogenicity and restricted tissue localization make them valid candidates for developing specific immunotherapy procedures. Earlier studies defined common features of these antigens: *(i)* their restricted expression profile, *(ii)* the presence of multi-gene families, *(iii)* their mapping to the X chromosome, and *(iv)* the immunogenicity in cancer patients. Subsequently, other characteristics shared by this group of genes and their products have been identified, while also recognizing exceptions to each rule. Additional features incorporate heterogeneous expression in cancer, correlation of mRNA expression with tumour progression and higher malignant potential, and activation by hypomethylation and/or histone deacetylation[7].

The expression frequencies of several CTA have been determined in various cancers of unrelated histologic origin, although the actual information on the expression in brain tumours remains scarce. Syed *et al*[8] have, recently, analysed the expression of CTA in malignant glioma tissue and primary glioma cell lines and compared with normal brain specimens and meningioma. The antigens most frequently expressed included melanoma-associated antigen-3 (MAGE-3) (22%), MAGE-1 (16%) and CT-7 (11%). The remainder of antigens demonstrated a pattern of low expression frequency (<10%). NY-ESO-1 was the only CTA demonstrated and seen in 12 per cent of meningioma tissue specimens. In 2006, Grizzi *et al*[10] investigated the immunolocalization of Sperm protein 17 (Sp17) in specimens of NS malignancies, to establish its usefulness as a target for tumour-vaccine strategies. Sp17 was previously entitled as a CTA in ovarian cancer, multiple myeloma and other malignancies[11]. A number of neuroectodermal (21%) and meningeal tumours (4%) expressed Sp17[10]. In addition, it was found that the expression pattern was

heterogeneous in all of the positive tissue specimens, and did not correlate with the degree of malignancy. Although, these results showed the immunolocalization of Sp17 in a proportion of NS tumour cells, but not in their non-pathological counterparts, the frequency of expression and non-uniform cell distribution of Sp17 suggested that it cannot be used as a unique CTA in NS cancers[10]. Sahin *et al*[12] investigated the expression of seven CTA genes (*i.e. MAGE-3, NY-ESO-1, HOM-MEL-40/SSX-2, SSX-1, SSX-4, HOM-TES-14/SCP-1,* and *HOM-TES-85*) in human brain cancers, and concluded that a majority of oligoastrocytomas and astrocytomas might be amenable to immunotherapeutic interventions, although the identification of additional TAA should allow for the development of widely applicable polyvalent glioma vaccines. Bodey *et al*[13] analysed the expression of *NY-ESO-1* in a series of childhood intracranial primary brain cancers, and found NY-ESO-1 in 10 to 40 per cent of the neoplastic cells of cerebellar primitive neuroectodermal tumour/ medulloblastoma that were examined and in <10 per cent of the tumour cells in high-grade anaplastic astrocytomas. They concluded that antigen-directed immunotherapy could target CTA, primarily those expressed at higher frequency[13].

It is now accepted that interpretation and comparison of the results of clinical trials using immunotherapy against brain tumours remain difficult because of variability in study design, therapeutic approach, immune endpoints measured, and patient eligibility criteria[14,15]. Though several CTA have been recognized, their expression in cancers has mainly been studied at the level of gene expression and gene level measurement by reverse transcriptase-polymerase chain reaction (RT-PCR) analysis and the quantitative real-time PCR (qrt-PCR) technology[16]. However, the information provided by these techniques is limited by the fact that the phenomena observed at each level of anatomical organization (*i.e.* gene, cell, tissue, organ, system or apparatus and the organism as a Whole) have properties that do not exist at a lower or higher level. RT-PCR and qrt-PCR may offer a satisfactory qualitative/quantitative description of small-scale structures, but this is likely to be irrelevant when it comes to large-scale features[16].

Brain tumours consist of a complex set of cells that differ in clinically phenotypic features[2,17]. The term ''heterogeneity'' defines the presence of sub-clones of cancerous cells with different genetic aberrations that mediate divergent biology and define the natural history of that particular tumour[18]. This phenotypic heterogeneity is a result of the interplay between genetic and non-genetic factors that shape cellular phenotypes[19]. The high number of cell cycles required for the formation of "macroscopic" tumours and the increased mutation rates allow for substantial genetic diversification of a tumour population. This phenotypic plasticity is what primarily determines the self-progression of neoplastic disease and its response to therapy[20]. Individual cells from a clonal cell population respond differently to the same stimulus, some not responding at all. It is known that in a heterogeneous population, patients may display a multiplicity of genetic variations that respond differently to a given medical intervention[18]. The same treatment could be of benefit to some patients yet harmful to others. Each cancer therapy can be viewed as a filter that removes a subpopulation of cancer cells that are sensitive to this treatment while allowing other insensitive subpopulations to escape. These considerations, in conjunction with the complexity of tumour-host interactions determined by an array of immune mediators expressed in the tumour microenvironment might partially explain the limits of current immunotherapeutic strategies[14]. Additionally, local non-cancer cells influence both tumour progression and outcome, illustrating the complexity of tumour environment. It is indubitable that a system level-based approach for validating the appropriateness of using CTA is now imperative to develop efficacious and less toxic immunotherapeutic strategies against brain cancers[16]. The system should includes the following key-points: *(i)* Discriminating the cell types expressing the candidate CTA; *(ii)* Discriminating the candidate CTA's sub-cellular localization; *(iii)*Mapping candidate CTA expression in all of the organs making up the apparatuses; *(iv)* Mapping candidate CTA expression in all of the apparatuses making up the human system; *(v)* Estimating the percentage of natural cells and their neoplastic counterparts expressing the candidate CTA; and *(vi)* Evaluating the dynamics of candidate CTA expression at the level of the cell cycle, the physiological status of the organism and the process of ageing.

Additionally, a clearer distinction must be made between *in vitro* laboratory results (*i.e.* the discovery and validation of TAA) and their *in vivo* validation, and it is necessary to adopt a more complete experimental approach that forcefully includes both morphological (*i.e.* immunohistochemical experimental methods) and molecular techniques.

Since our understanding of human cancer is still limited and pre-clinical models have shown a discouraging propensity to fail when applied to

humans, a new way of thinking is strongly needed that unites physicians, biologists, mathematicians and epidemiologists, to develop a better theoretical framework of brain tumour development, progression and tumour-host interactions.

It is indubitable that intra-tumour heterogeneity may explain the difficulties encountered in the validation of oncology biomarkers owing to sampling bias, contribute to Darwinian selection of pre-existing drug-resistant clones, and predict therapeutic resistance. As stated by Sampson *et al*[15] the heterogeneity of malignant brain tumours may limit the effectiveness of vaccinations that target only one TAA (*i.e.* epidermal growth factor receptor variant III, EGFRvIII). Vaccines that target only one antigen may not target all tumours or all cells comprising a tumour and may, therefore, select for the survival and proliferation of those cells that do not express the targeted antigen. This may ultimately limit this potentially promising strategy. Although this study demonstrates the possible benefits of vaccination with a peptide that contains a tumour-specific epitope, there remain various issues that must be addressed to optimize this therapeutic modality[15].

The use of an integrative approach will probably reduce the notable fragmentation of the biological information in the post-genomic era, and will facilitate a more accurate transfer of the acquired knowledge from "bench to the bedside". This way of thinking may help to clarify concepts, categorize the amount of biological knowledge, and suggest alternative approaches to discover new biomarkers with potential clinical value.

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