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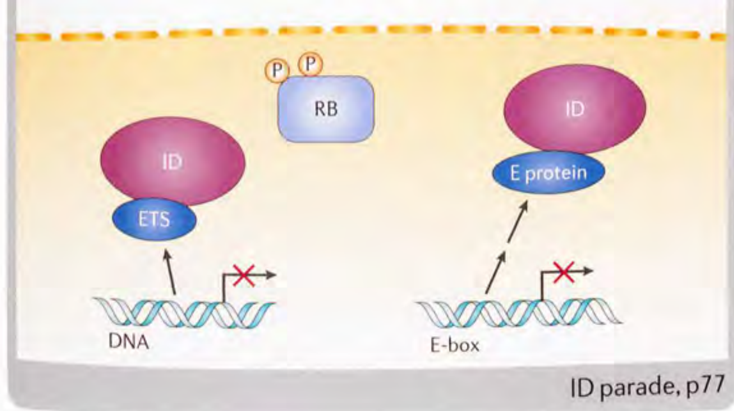

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Anna Lasorella, Robert Benezra and Antonio Iavarone

Inhibitor of DNA binding (ID) proteins are transcriptional regulators that control the timing of cell fate determination and differentiation in stem and progenitor cells. The ability of ID proteins to function as central 'hubs' for the coordination of multiple cancer hallmarks is establishing them as therapeutic targets and biomarkers in specific types of human tumours.

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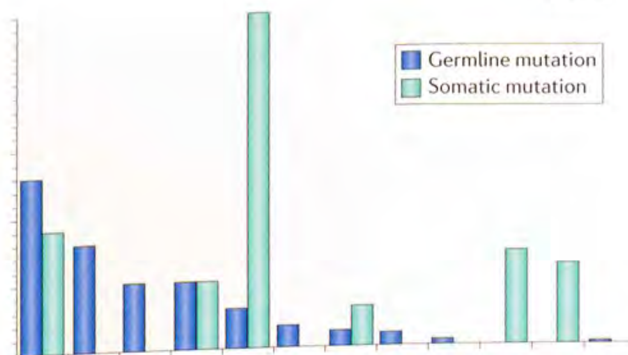

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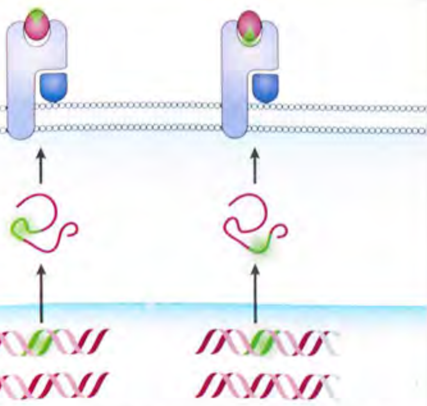

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TIMELINE

Tumour antigens recognized by T lymphocytes: at the core of cancer immunotherapy

Pierre G. Coulie, Benoît J. Van den Eynde, Pierre van der Bruggen and Thierry Boon

Abstract | In this Timeline, we describe the characteristics of tumour antigens that are recognized by spontaneous T cell responses in cancer patients and the paths that led to their identification. We explain on what genetic basis most, but not all, of these antigens are tumour specific: that is, present on tumour cells but not on normal cells. We also discuss how strategies that target these tumour-specific antigens can lead either to tumour-specific or to crossreactive T cell responses, which is an issue that has important safety implications in immunotherapy. These safety issues are even more of a concern for strategies targeting antigens that are not known to induce spontaneous T cell responses in patients.

Cancer immunotherapy that involves the deliberate use of the adaptive immune system to reject tumours or to prevent their recurrence is gaining momentum. Interesting clinical results have been obtained using cancer vaccines, adoptive T cell therapies and antibodies that stimulate the activity of T lymphocytes. Moreover, increasing evidence suggests that adaptive immunity contributes to the long-term clinical benefits of anticancer treatments such as chemotherapy and radiotherapy. At the core of these clinical developments lies the fact that cancer patients can produce T lymphocytes that recognize tumour-specific antigens. The first human tumour-specific antigens that were recognized by T cells were discovered about 20 years ago (FIG. 1 (TIMELINE)). Considering the increasing number of clinical studies that rely on the presence of tumour-specific antigens that are recognized by T cells, it is worth summarizing the key steps that led to their identification, and it is worth describing the genetic processes that result in their presence on tumour cells. A proper understanding of the factors that affect the degree of specificity of the T lymphocyte response against tumour antigens is essential to aid the design of immunotherapy strategies that are not only efficient but also free of adverse side effects.

Identification of mouse antigens

Initial controversy about the existence of tumour rejection antigens. From 1940 to induced with oncogenic viruses showed that the immune system could reject these tumours following the recognition of viral antigens[1]. The first evidence that mouse tumours that were not induced by viruses could also be recognized by the immune system was obtained by Gross and colleagues in 1943 (FIG. 1 (TIMELINE)). They induced tumours in mice through the use of chemical carcinogens and then resected these tumours. These mice were able to reject the same tumour cells on subsequent exposure[2]. Mice that were immunized with lethally irradiated tumour cells were similarly protected. These results were confirmed by other groups[3], and in the 1960s it became widely accepted that mouse tumour cells and therefore possibly human cancer cells could be recognized by the immune system.

In sharp contrast, in 1976, Hewitt[4] reported that a similar analysis carried out with spontaneous tumours that developed in mice failed to produce any evidence of immune control. He concluded that mouse tumour antigens were artefacts that were induced by the chemical treatment used to induce experimental tumours and were therefore unlikely to be present on human tumours.

In the 1970s, we treated a mouse teratocarcinoma cell line *in vitro* with a strong mutagen, and we showed that many cell clones that were derived from the mutated population were incapable of forming progressive tumours when injected into rejected by an immune response directed against new antigens that were different for every variant (tum^{-} antigens). Remarkably, mice that had rejected tum^{-} variants were also protected against a subsequent injection of the parental tumour cells[7], even though this teratocarcinoma was non-immunogenic, similar to the tumours that were described by Hewitt. We concluded that an efficacious response against the tum^{-} antigens had an additional effect: it triggered a response against antigens that were present on the original tumour but that were apparently non-immunogenic on their own. In collaboration with Hewitt, we treated cells from spontaneous tumours with mutagens to obtain tum^{-} variants, and we observed that these variants were also capable of inducing immune protection against the parental tumours[8]. This showed that spontaneous mouse tumours do express tumour antigens, albeit poorly immunogenic ones. We became convinced that human tumours might also be susceptible to immunological treatment and that we should first identify the nature of the rejection antigens that were observed on the mouse tum^{-} variants and their parental tumour.

Molecular identification of antigens recognized by T lymphocytes on mouse tumours. After the discovery of T lymphocytes in the 1960s[9,10], their essential role in graft rejection and tumour rejection was soon realized[11,12]. In the tum^{-} system, we observed that adoptive transfer of T cells, which were collected from mice following rejection of a tum^{-} variant, protected irradiated mice against the growth of the same variant. This clearly indicated the involvement of T lymphocytes in the tum^{-} phenomenon. Accordingly, for several years, we attempted to obtain specific cytolytic T cells that were directed against tum^{-} variants; this was unsuccessful. We eventually turned to the P815 mastocytoma cell line, which proved to be remarkably easy to cultivate and to clone because it proliferated in suspension. Tum^{-} variants were readily obtained for this cell line[13]. Moreover, excellent cytotoxic T lymphocyte (CTL) responses were obtained that showed clear specificity for each tum^{-} variant[14].

We then benefited from a major advance in the CTL field: microcultures could be derived from a single CTL by repeated stimulations with irradiated target cells in the presence of a T cell growth factor that was later identified as interleukin-2 (IL-2)[15]. These clonal CTL cultures could be expanded to large numbers and could be

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clones that were directed against a single antigen proved to be crucial for a rigorous analysis and dissection of the antigens recognized by T cells on several target cells.

With great help from Cerrotini and his group, who had had a prominent role in these developments, we obtained stable CTL clones that killed the stimulatory tum^{-} variant but not the other tum^{-} variants nor the parental tumour cells[16]. These CTL clones clearly recognized a tum^{-} antigen that was induced by the mutagen treatment. Other CTL clones killed both the tum^{-} and parental cells, evidently recognizing an antigen that was present on the original P815 tumour cells. That these antigens were genuine rejection antigens was shown by the *in vivo* observation that some tumours progressed, then nearly completely regressed, then progressed again. These 'escaping' tumours had invariably lost the antigen that was recognized by one of the CTL clones[17]. This was true not only for tum^{-} antigens but also for antigens that were present on the parental tumour[18]. In fact, these and other studies that were carried out in the early 1980s formally showed the reality of tumour immune surveillance and the occurrence of tumour escape after immune selection[19], which is a process that was recently renamed 'immunoediting' (REF. 20). Although immunoselected tumour variants were resistant to some CTL clones, they were still sensitive to others. A detailed analysis of a panel of such variants led to the conclusion that CTLs recognized several (typically less than ten) distinct antigens on a given tumour[17,18].

The next step was to define the molecular nature of these antigens. The only available tools were the stable CTL clones. The exact molecular nature of the antigens that were recognized by CTLs was unknown at that time. However, the notion that antigens are recognized by T lymphocytes in association with major histocompatibility complex (MHC; human leukocyte antigen (HLA) in humans) molecules had been known for a decade[21]. In 1986, Townsend showed that antiviral CTLs recognized small peptides of eight to ten amino acids, which were derived from a viral protein and presented at the surface of infected cells in association with MHC class I molecules[22]. Soon thereafter, an excellent crystallographic study showed that MHC class I molecules present small peptides in a groove that is located at the surface of the molecule[23]. We now know that these peptides are produced by partial digestion of the parental protein, mainly through the proteasome machinery (BOX 1). These peptides then become associated with the MHC class I molecule and are displayed at the cell surface following a process known as the 'antigen processing pathway' (FIG. 2).

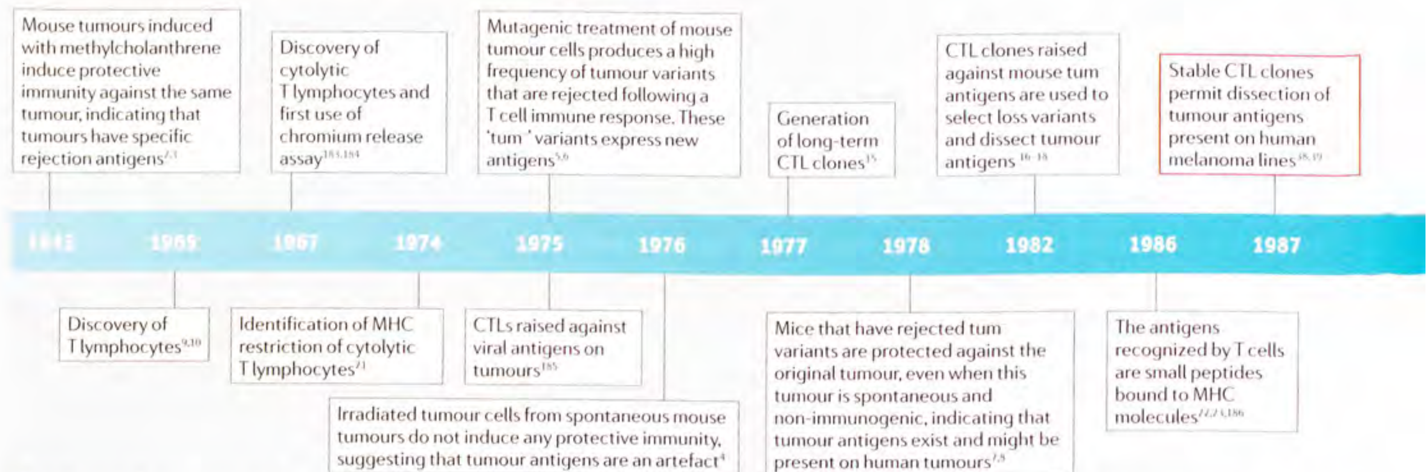
To identify our antigens, we used a genetic approach that aimed to clone the gene encoding the antigen. Once again, the P815 cells were invaluable, as we were able to select a highly transfectable variant named P1.HTR[24]. We transfected P1.HTR with a gene library derived from cells that expressed a given tum^{-} antigen and, using the relevant CTL clone, we identified a transfectant that expressed the antigen. The encoding gene was retrieved from the transfectant and sequenced.

The first gene that encoded a tum^{-} antigen was cloned in 1988 (REF. 25). It encoded a ubiquitous protein of unknown function. Crucially, the coding region contained a mutation that changed one amino acid in the protein. Small peptides that contained the mutated residue were shown to sensitize parental P815 cells to CTL-induced cell death, whereas corresponding wild-type peptides did not[26]. We concluded that the antigen was a complex between the mutated peptide and the presenting MHC class I molecule.

The identification of two other tum^{-} antigens[27,28] indicated that each of them also resulted from a point mutation in a ubiquitously expressed gene. Each mutation created a new antigenic peptide. In some cases, the mutation enabled the peptide to bind to the groove of the presenting MHC molecule. In other cases, the mutation created a new epitope in a peptide that was already bound to MHC, but the wild-type peptide was not recognized by T cells because of central tolerance (FIG. 3a). Even though tum^{-} antigens were artificially induced by mutagen treatment, their identification established the principle that rejection antigens can result from mutations in ubiquitously expressed genes. These results showed for the first time the occurrence of a process of immune surveillance of genome integrity.

We then set out to identify the tumour rejection antigen that was present on the parental mouse tumour P815. This time, the identified antigen, which was named P1A, did not result from a mutation. The antigenic peptide corresponded to the normal

Timeline | **Milestones in the discovery of tumour rejection antigens**



Black boxes refer to discoveries that are related to mouse tumours; red boxes refer to discoveries that are related to human tumours. Observations and discoveries that are related to viral antigens are not included. BAGE, B melanoma antigen; CTAG, cancer/testis antigen; CTL, cytotoxic T lymphocyte; GAGE, G antigen; *MAGEA1*,

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