Therapeutic Delivery

Biopharmaceuticals and gene vectors opening new avenues in cancer immune therapy

"Now, that we have a better understanding of the immune system, its interplay with malignant diseases and have proper tools at hand, it should make us optimistic to enter a new phase in the fight against cancer.."

Keywords: cancer • cancer gene therapy • immune checkpoint inhibitors • oncolytic virus

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In recent years, use of biopharmaceuticals (such as antibodies, cytokines, nucleic acids) has experienced a real boost in many disease types, especially in the treatment of malignant diseases. Several oncologists have already started a swansong on conventional chemotherapeutics, which often, after being initially successful, fail due to the emergence of resistance mechanisms. Two major topics have emerged to be very promising for successful and sustained treatment of cancer *in vivo*. One is to harness the human immune system and specifically activate it to attack and destroy cancer cells. The other is the emerging knowledge on cancer initiating cells, also termed cancer stem cells (CSCs), and the development of specific therapies to eradicate them in a specific manner.

Our body is constantly faced with damaged cells due to exposure to radiation, chemicals, viral infections and other cancerogenics. It is the orchestrated action of the innate and adaptive immune system, which keeps the control, and tumor surveillance [1]. Only when several control mechanisms fail, tumor escape occurs. Hence, it seems logical to utilize immunological approaches for sustained cancer eradication.

First-generation of anticancer antibodies

As a major immunological spearhead in the treatment of cancer, monoclonal antibodies have demonstrated considerable potential. Currently, four of the top ten selling anticancer drugs are antibodies (rituximab, bevacizumab, trastuzumab, cetuximab) (http://www.medscape.com/viewarticle/826649), and there are still more to come. This first wave of antibodies is directed against distinct antigens overexpressed on tumor cells, which can also be found on non-malignant cells but at lower expression levels, like growth factor receptors (EGF-receptor, VEGF-receptor), or B-cell antigens (CD20). Besides affecting their target after binding, like inhibition of downstream signalling in case of EGF-R (Cetuximab), their distal Fc-part acts as a potent activator of antibody mediated cellular cytotoxicity (ADCC, [2]), complement activation and others. Albeit highly efficient in the beginning, such antibodies can also fail, mainly due to their high specificity. The most probable scenario is loss of the antigen, against which antibody is targeted. Also, the antigen expression might be not vital for a growth advantage of the cancer cell, and its functions can be taken over by other surface proteins. For example, triple negative breast cancer does not only lack oestrogen and progesterone receptor (making the cancer growth hormone independent), but also Her2/neu (erb-B2), the target for Trastuzumab.

Second-generation of anticancer antibodies

A major defense mechanism of solid cancers is the local generation of an immune suppressive environment, either by the overexpression of immune-suppressing receptors, or the secretion of immune suppressive cytokines.

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Recently, a novel generation of antibodies entered the clinics, which can specifically interfere with distinct modulators of the immune system associated with tumor mediated generation of immune suppressive environment. To prevent overshooting immunological activities, many immune cells bear surface molecules, which upon binding with an appropriate ligand/receptor downregulate their activity. For example CTLA-4 expressed on T-cells blocks their stimulation, when binding to the ligand B7 expressed on the surface of antigen-presenting cells. The PD-1 receptor is expressed on a broad range on immune cells, and interaction with its ligands PD-L1 or PD-L2 leads to immune suppressive effects [3]. While PD-L2 expression is restricted to antigen-presenting cells, PD-L1 is upregulated in many tumors promoting an immune-suppressive environment [4]. For both targets, blocking antibodies have been developed and are already clinically applied for the treatment of melanoma and other solid cancers [5]. Ipilimumab (Yervoy®) is a humanized antibody approved for the treatment of advanced melanoma blocking CTLA-4. Nivolumab and pembrolizumab bind to PD-1, and are used in lung cancers and melanoma. Similarly, CD47, a strong 'don't eat me' signal, is ubiquitously expressed on solid tissues and blood cells including erythrocytes [6]. Its major binding partner, SIRPα, can be found on macrophages, and upon CD47/SIRPα interaction potential phagocytosis is halted. CD47 has been reported to be overexpressed on a broad range of cancers, contributing to their immune suppressive action. Interestingly, CD47 is also upregulated on cancer-initiating cells (also termed cancer stem cells [CSCs]), which are extremely difficult to treat by conventional therapies due to very slow mitotic rate (here a recent report on a high rising CSC conference [7]). A highly interesting preclinical study was presented recently, by combining a SIRPα-derived CD47 blocking protein with rituximab, which resulted in complete tumor eradication [8]. First CD47 targeted clinical trials are on the way uniting the treatment options for two major potential 'achilles heels' of cancer, the immunological approach and CSC, like the CAMELLIA trial for treating leukemia [9] and solid cancers [10].

Cytokines against immune suppression

TGF-β, for example, reduces the activity of immune effector cells, and promotes activity of immune suppressing cells, like T_{reg} [11]. Here, cytokines can be applied to specifically counteract on this immune suppression. Major immune stimulatory cytokine based drugs on the market are TNF-α, IFN-α and IFN-β, interleukines (mostly IL-2) and GM-CSF (for a comprehensive overview on cytokines used as anticancer therapeutics and their biological function see [12]). TNF-α, for example, is a highly potent cytokine, either for direct killing of cancer cells or in combinations with chemotherapeutics. TNF-α can directly destroy tumor cells, which express death receptors, triggering their rapid apoptosis/necrosis [13]. Nevertheless, this direct action is often counteracted by tumors due to NFκB upregulation, which equalizes the TNF-α effect. Another effect of TNF-α is to increase the permeability of the (tumor) endothelium. Here, it can potentially help to reduce the interstitial pressure of solid tumors and boost drug accumulation. Nevertheless, TNF-α as a protein can also only be applied locally due to dose limiting, potentially fatal side effects. The protein, marketed as Beromun® by BoehringerIngelheim, is applied by the so-called isolated limb perfusion method, as otherwise therapeutically active protein doses results in live threatening internal bleeding and inflammatory reactions, but excludes treatment of organ located lesions. While IL-2 and IFN-α, for example, can be applied at therapeutically active doses, toxic side effects prohibit the use of cytokines having a high potential for anticancer treatment. IL-12, for example, secreted by macrophages and dendritic cells, is a potent activator of immune effector cells, like T-helper cells, naive T-cells and natural killer (NK) cells and bridges the innate and the adaptive immune system (for a comprehensive overview of IL-12-based therapies, see [14]). Also here, intravenous IL-12 treatment causes severe side effects, especially when triggering secretion of other cytokines which led to hematological toxicities.

Cytokines, gene therapy approaches

While the cytokine itself is highly toxic (including side effects), applying a gene vector to deliver the inactive 'prodrug' DNA to the tumor and have it specifically expressed on site, can be a suitable approach. We have developed a gene carrier for selective delivery of *TNF*-α gene into tumors [15], where we could unambiguously demonstrate, that tumor-restricted TNF-α expression promoted accumulation of liposomal doxorubicine (Caelyx®). This resulted in a synergistic effect reducing total tumor burden in a disseminated liver metastases model of human colorectal cancer. *TNF*-α gene therapy is also pursued in clinical trials: while a *TNF*-α encoding adenoviral vector (TNFerade®) in combination with 5-fluorouracil (5-FU) failed to show an improved response compared with 5-FU alone (Phase III trial with local application in pancreatic cancer, [16]), other trials are on the way using modified gene vectors. As TNFerade belongs to an early generation of adenoviral gene vectors (non-replicating), its distribution within the tumor is limited, whereas oncolytic vectors (see below)

can be the better option. Already 20 years ago, *IL-12* gene therapy was evaluated enabling localized, sustained release of the cytokine [14]. Although initial clinical trials with IL-12-encoding gene vectors as monotherapy were less effective [17] (mainly due to limited transgene expression and spread within the tumor), IL-12-based gene therapy has encountered a revival using novel gene vectors and delivery strategies: local application of plasmid in combination with electroporation enables high local cytokine concentrations, and when combined with chemotherapeutics, very promising data were obtained, for example, in a clinical study in companion dogs [18]. Currently, an oncolytic vector (see below) encoding for IL-12 is used in a Phase I clinical trial in glioblastoma patients [19].

Oncolytic viral vectors, cytokine armed

The initial idea, when using recombinant viruses as gene vectors, was to make them as safe as possible, which included the removal of most elements which could potentially lead to (unwanted) viral replication. It took some time to recall the intrinsic features of certain viruses, namely to replicate more or less selectively in cancerous tissue while destroying it. Now we have technologies at hand, which allow the generation of oncolytic vectors, which are safe enough to ensure highly tumor selective replication, and the possibility to additionally 'arm' them for even more potent immune stimulation. Oncolytic vectors offer several advantages, as they combine immune- and gene therapy in an almost perfect way [20]. Being designed to replicate only in cancer cells with distinct deregulated signalling pathways, they can be additionally equipped with expression cassettes for therapeutically active transgenes, like cytokines. Their activity is based on several pillars: after infecting and lysing their target cells, release of reactive oxygen species and immune stimulating cytokines is triggered. Further immunostimulation is achieved by release of danger signals, but also of otherwise inaccessible, intracellular tumor antigens, which could even result in a memory effect making the oncolytic vector even a cancer vaccine. A current success story is the Herpes-virus based oncolytic vector Talimogene Laherparepvec (T-VEC). Besides its tumor-cell specific lysis, T-VEC encodes for GM-CSF. Recently, it has been approved both by the US FDA and the European EMA for the local treatment of melanoma [21]. The approval of T-VEC is the latest development in the success story of immune-stimulating vectors, and there are for sure more to come.

Future perspective

We have seen the development of armed oncolytic vectors moving into clinical application, and at the same time the application of a novel generation of antibodies, which can break immune tolerance by blocking immune-suppressive pathways harnessed by cancers. The simplest and most straightforward way would be a combination therapy, or even arming oncolytic vectors with gene products which could block the above-mentioned mechanisms. Preclinical studies with measles virus combined with CTLA-4 and PD-1 blocking antibodies already demonstrated a synergistic effect, and a clinical application of such a schedule is feasible [22]. Limitations due to the macromolecular nature of biologicals and gene vectors can be overcome by selectively increasing the permeability of tumor tissue, like with cytokines (see above), or other proteins, which can selectively open the endothelium and further promote the spread of oncolytic vectors [23]). We should recall that cancer is in principle a disease that arises due to a malfunction of our immune system. Even more than a century ago, it was recognized by William Coley that infections can 'melt away' tumors [24], and 60 years ago it was recognized that viruses have a certain preference to replicate in tumor cells [25]. Now, that we have a better understanding of the immune system, its interplay with malignant diseases and have proper tools at hand, it should make us optimistic to enter a new phase in the fight against cancer.

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