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Review Article

Oncolytic adenoviruses as a therapeutic approach for osteosarcoma: A new hope

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ABSTRACT

Osteosarcoma is the most common bone cancer among those with non-hematological origin and affects mainly pediatric patients. In the last 50 years, refinements in surgical procedures, as well as the introduction of aggressive neoadjuvant and adjuvant chemotherapeutic cocktails, have increased to nearly 70% the survival rate of these patients. Despite the initial therapeutic progress the fight against osteosarcoma has not substantially improved during the last three decades, and almost 30% of the patients do not respond or recur after the standard treatment. For this group there is an urgent need to implement new therapeutic approaches. Oncolytic adenoviruses are conditionally replicative viruses engineered to selectively replicate in and kill tumor cells, while remaining quiescent in healthy cells. In the last years there have been multiple preclinical and clinical studies using these viruses as therapeutic agents in the treatment of a broad range of cancers, including osteosarcoma. In this review, we summarize some of the most relevant published literature about the use of oncolytic adenoviruses to treat human osteosarcoma tumors in subcutaneous, orthotopic and metastatic mouse models. In conclusion, up to date the preclinical studies with oncolytic adenoviruses have demonstrated that are safe and efficacious against local and metastatic osteosarcoma. Knowledge arising from phase I/II clinical trials with oncolytic adenoviruses in other tumors have shown the potential of viruses to awake the patient´s own immune system generating a response against the tumor. Generating osteosarcoma immune-competent adenoviruses friendly models will allow to better understand this potential. Future clinical trials with oncolytic adenoviruses for osteosarcoma tumors are warranted.

1. Osteosarcoma: the disease

Compared to other tumors, bone cancers are relatively rare cancers with an average incidence less than 1 per 100,000 person-year [1,2]. Bone cancers encompass different types of tumors, such as Ewing sarcoma and chondrosarcoma, but the most frequent among them is the osteogenic sarcoma, also known as osteosarcoma (OS), which comprises a 20–40% of total new diagnosed bone cancers [2,3]. OS tumors are characterized by the overproduction of an aberrant osteoid matrix surrounding malignant spindle cells that leads to a high risk of fracture within the affected bone [3,4]. Most of OS primary tumors are located in the metaphysis of the long bones in both upper and lower limbs, where there are developed about a 75–85% of the tumors [5,6]. However, the main issue of OS tumors is their high spread of malignant cells along the organism and as a consequence most of the patients present metastasis at the diagnosis, thus worsening the prognosis of the disease.

The incidence of OS shows a bimodal age distribution with a major peak observed during childhood and puberty, and a second minor peak that appears in the elderly [2,5,7]. There are also gender related differences in the epidemiology of OS, with an overall higher incidence in males [1].

Although there is still a little knowledge about the etiology of OS, the incidence characteristics described above, as well as, the predominant location of these tumors in limbs suggest a relationship between bone growth and the development of the disease in young patients [8–10]. On the other hand, OS in adults often appears as a secondary malignancy [11] and the occurrence of these tumors has been linked to some predisposing syndromes like Li-Fraumeni syndrome [12].

2. Osteosarcoma: treatment, survival and further needs

Before the arising of chemotherapy, the only treatment available for OS was the amputation of the affected limb, even though the result was

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a poor survival rate below 20% of the patients [13]. Nowadays, the standard protocol for high-grade OS includes the surgical resection of the primary tumor and its metastases in combination with both neoadjuvant and adjuvant therapies using different cocktails of high-dose methotrexate with leucovorin rescue, adriamycin, cisplatin and cyclophosphamide or ifosfamide [13]. As a result, this regimen has increased the survival rate in the last 50 years up to a 60–70% of the patients, as well as the evolution of imaging and surgery techniques has improved the limb-salvage interventions over the 90% [13,14]. However, with the current strategies the success of OS treatment has reached a plateau and the survival rate has not been increased in the last 30 years. Moreover, besides the primary location within long bones, OS is a highly metastatic tumor releasing invasive malignant cells that migrates through the organism, thus leading to the development of metastases in lungs [15–17] and, unfortunately, in those patients with metastatic OS the survival rate drops to a poor 30% [18]. The lack of progress in the fight against OS is associated with the presence of drug-resistant tumor cells [19]. In addition, some OS tumors cannot be completely removed due to their location, especially those affecting the axial skeleton.

On the other hand, the aggressiveness of the chemotherapeutic treatment is associated with severe side effects including oocyte destruction and infertility, hearing impairment, heart failure, hepatotoxicity and nephrotoxicity [20–26].

For all these reasons, it is urgent to implement new therapeutic approaches against osteosarcoma which allow improving the survival rate as well as reducing the side effects. In the last years virotherapy has emerged as a true potential strategy in cancer medicine. Currently several viruses are being tested as anticancer agents, and among them we will focus on oncolytic adenoviruses.

3. Adenoviruses: structure and viral cycle

Although there are dozens of different adenovirus described, all of them share a common architecture. Adenoviruses are non-enveloped viruses with an icosahedral capsid composed by up to 7 different structural proteins. However, considering the scope of this review the most relevant viral protein is the fiber due to its role in adenovirus tropism. The fiber is a trimeric protein which is located on each of the 12 vertices of the virion and protrudes from the capsid as an elongated antenna [27]. The fiber protein is composed of three domains (Fig. 1A): 1) a proximal tail domain that anchors the protein to the capsid; 2) a distal globular knob domain that recognizes and binds to the cellular receptor, and 3) a fibrous shaft domain which keeps the knob away from the capsid, thus avoiding steric repulsion during the virus-cell interaction.

On the other hand, the adenovirus genome is composed by a single linear dsDNA molecule of about 36 kb long and encodes for more than 40 gene products clustered into different transcription units, which in turn are named as early, intermediate or late depending on if they are transcribed before, during or after the DNA replication, respectively [28].

Regarding the viral cycle, first the adenovirus recognizes its specific receptor on the cell surface triggering its internalization. Once inside the cell the virus migrates through the microtubules and introduces the viral genome inside the nucleus. There the E1A gene is expressed immediately from the adenovirus genome. The E1A protein is able to bind pRb [29–31], releasing the transcription factor E2F and thus the arrest in the cell cycle. The release of E2F also triggers an orchestrated activation of the viral genes that eventually will lead to the generation of new virions, the lysis of the infected cell and the spread of the viral progeny (Fig. 1B).

Additionally, the E1B-55k viral protein is also transcribed during the initial stages of the infection and inhibits the tumor suppressor p53 to avoid its counterbalance effect in cell cycle progression as well as the E1A-induced apoptosis mediated by p53 [32,33].

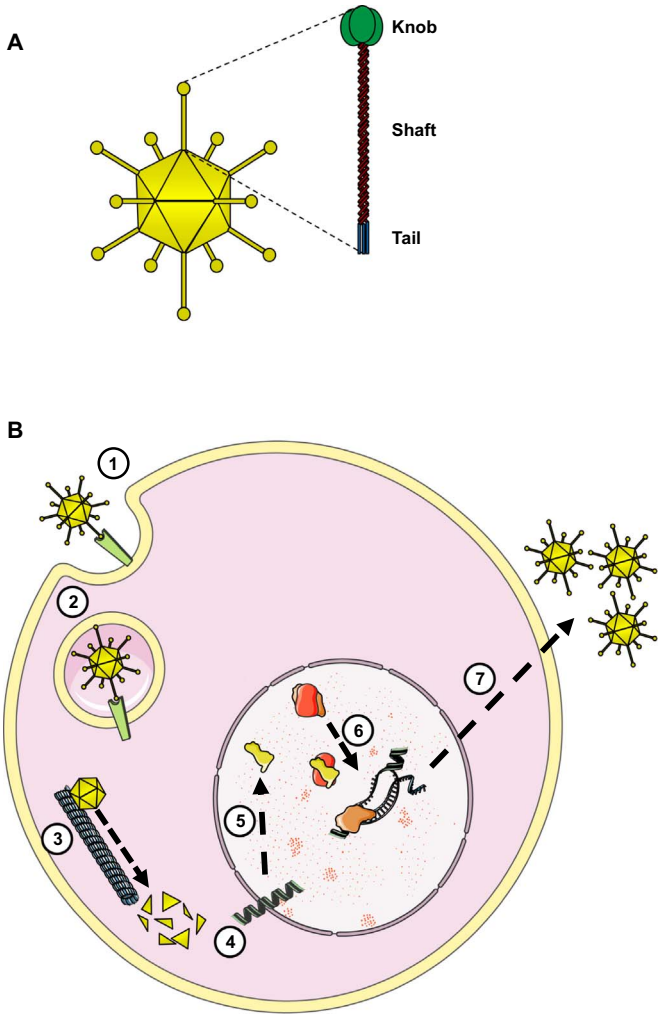

Fig. 1. A) Illustration showing the location of the fiber protein on the adenovirus capsid, as well as the three structural domains of the fiber. B) Schematic adenovirus viral cycle. The adenovirus recognizes its specific receptor (1) triggering its internalization inside the cell (2). Then the virus migrates through the microtubules (3) and introduces the viral genome inside the nucleus (4). The E1A gene is expressed immediately (5) the E1A protein binds pRB releasing the transcription factor E2F and thus the cell cycle arrest (6), which in turn will promote the expression of viral proteins and the genome replication, obtaining the viral progeny (7).

Among the different species and serotypes described, the human adenovirus serotype 5 (Ad5) is, by far, the most studied adenovirus. The Ad5 is a very common virus that has tropism for the upper respiratory tract and usually causes just flu-like mild infections. Nevertheless, besides its role as a natural human pathogen, in the last three decades the interest of this virus has been boosted because of its potential utility in different biomedical fields such as gene therapy, vaccination and virotherapy [34–37]. Although there are some studies using other adenovirus serotypes, the vast knowledge about the biology of the Ad5, as well as its relatively harmless behavior are the main reasons that places this serotype as the reference adenovirus in biomedicine.

In this article we will review briefly the state-of-the art of Ad5 derivatives as therapeutic agents in the field of virotherapy and how are they being tested to treat osteosarcoma.

4. Oncolytic adenoviruses as therapeutic tools: tumor specificity

As described before, adenoviruses infect their target cells and subsequently replicates inside the nucleus and lyses the cells to spread