ELSEVIER

Intracellular caspase-modulating chimeric antigen receptor

Scott Tarone

Tinton Falls, NJ, USA

In this review, a targeted cancer therapeutic is proposed providing direct targeting of tumor-specific or -associated antigens from within malignant cells: (i) with an antibody-based biological agent; (ii) utilizing a constitutively active apoptosis effector that is inhibited or nonfunctional until the antibody binds its agonist. Two possible configurations to achieve this end are provided. One embeds an scFv control into the apoptosis-inducing effector using an intein and the second employs a zinc-finger-based targeting and activating mechanism based on an approach known as sequence-enabled reassembly of proteins. Although the latter might provide a broader range of targets, because zinc fingers bind directly to DNA rather than transcribed protein, this review focuses on the former owing to the large body of clinical data available.

Targeting within the cell

Cancer immunotherapy with chimeric antigen receptors (CARs) involves the transfection of autologous T cells with receptors specific for a tumor antigen. The main requirement in the development of a clinically successful CAR is that this antigen is available on the malignant cell surface. CAR T cells have achieved great success in trials with complete remission observed in patients with chemorefractory chronic lymphocytic leukemia (CLL) and acute lymphoblastic leukemia [1,2]. However, there have been tragedies in other trials: one patient receiving erbB-2-targeted CARs experienced respiratory distress within 15 min and died four days later. In the case report investigators speculated that an on-target/off-tissue event occurred with the T cells releasing cytokines upon recognition of low levels of erbB-2 on lung epithelial cells [3].

The main requirement of CAR-based therapy has become its greatest obstacle because the search for suitable cell surface tumor targets has not yielded a group of tumor-specific antigens but rather those shared with normal cells. The most recent release on the growth of the T-cell-defined tumor antigen database reports its 6th selection criteria: 'a certain level of tumor- or tissue-specificity should be documented, as ubiquitous antigens do not qualify as tumor antigens' [4]. The potential for on-target/off-tissue serious adverse events (SAEs) remains as long as the targets are shared even when preferential binding has been observed. Even the highly successful CD-19-targeted CARs invariably result in chronic hypogammaglobulinemia. To mitigate this risk, a biological agent is proposed, hereafter it will be referred to as an intracellular caspase-modulating chimeric antigen receptor (iCCAR), to be delivered directly into the cytosol and subcellular compartments, which is capable of detecting conserved signature sequences in mutant proteins and, upon detection, directly inducing apoptosis (Fig. 1).

The antigen-binding domain

In the iCCAR, the antigen-binding domain is an scFv derived from a mAb engineered with specificity for a signature epitope within the sequence of mutant protein expressed in malignant cells of a given phenotype. In most cases, cells from malignant tissue from an afflicted individual will be sequenced to identify the epitope, thus requiring on-demand engineering of the applicable chimera.

However, this might not always be the case. Regarding oncogenes, the research suggests it is not the function of the protein product that is necessarily altered in oncogenic mutation but rather a change in transcription regulation, a substantial decrease in the ubiquitination and proteasome turnover rate or its active state changing from induced to constitutive that is the actual driver of oncogenesis [5,6]. From the latter two points, it seems plausible that the mutations should involve only a few sequence substitutions given that the protein itself is left functionally intact. Whether these 'hotspots' arise from single nucleotide polymorphisms (SNPs) or consistently aligned translocations, it appears that there could be epitopes that are common across cancer types. This could leave only one major question: whether or not a sufficient level of specificity is achievable to ensure off-target toxicity is not a probable outcome. The fact that it has been

E-mail addresses: starone@synapticsynthase.com, starone@comcast.net.

http://dx.doi.org/10.1016/j.drudis.2015.02.001

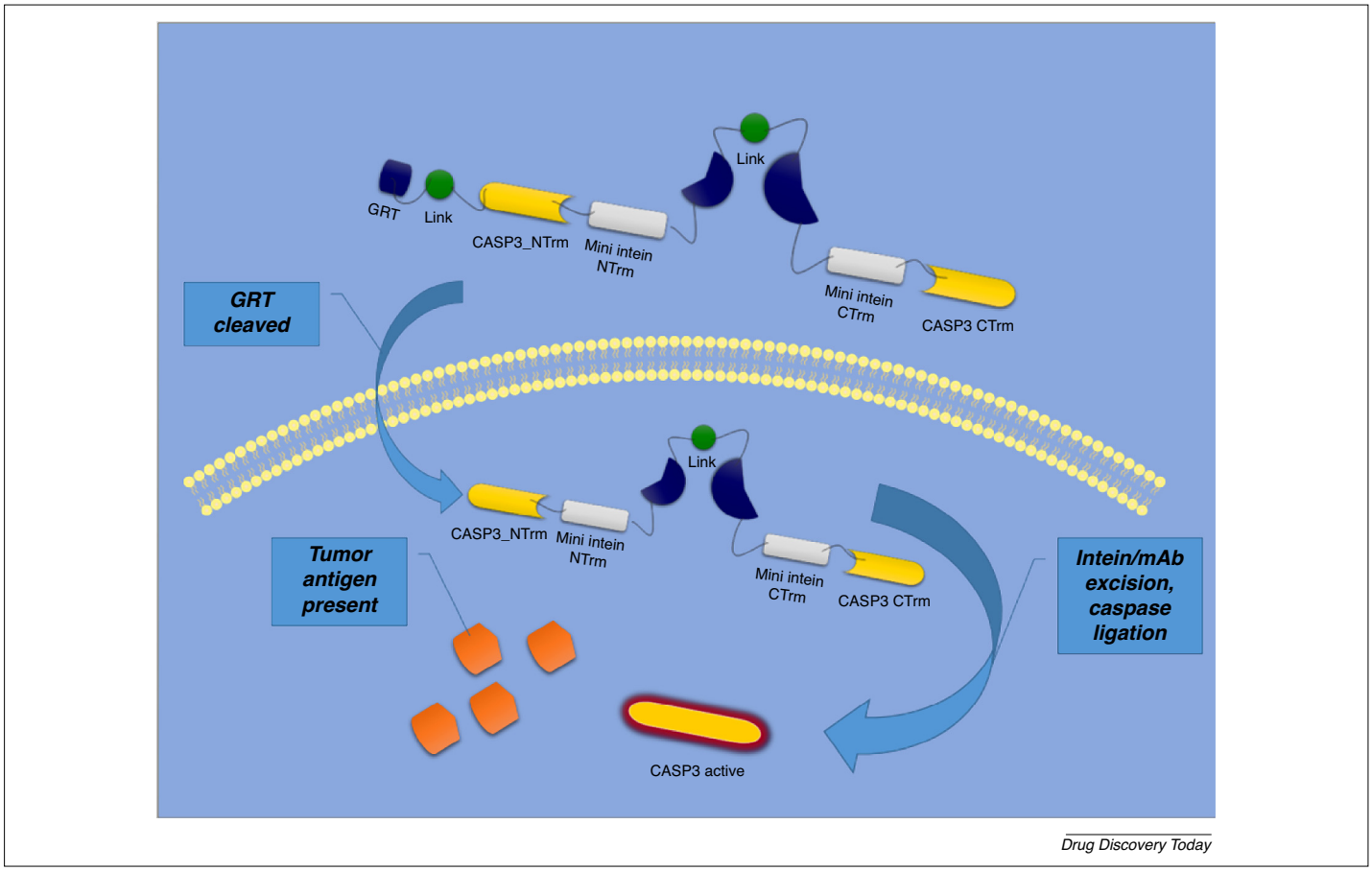

FIGURE 1

Intracellular Caspase-Modulating Chimeric Antigen Receptor (iCCAR) Delivery and Activation. Upon iCCAR entering the reductive intracellular environment guanidinium-rich transport is enabled. If the target antigen is present, mAb binding will induce extein excision and ligation of the constitutively active caspase-3.

reported that amino acids with charged side-chains can influence specificity by 1000-fold might hold the answer, and at the very least could help focus the search [7].

Further, although malignancies can involve a large number of mutated genes, it is becoming clear that there are a relative few that are crucial for survival and, of those, some have an active role whereas others are passive. That is, certain mutated genes encode products that drive oncogenesis and thus are required for survival and growth whereas others are not. This phenomenon, referred to as 'oncogene addiction', reveals that, although there might be many mutant genes in a malignant cell genome, the targeting of the product of just one mutant gene can lead to cell death. There have been many cases in humans and animals where this has been successfully demonstrated [5]. Given this, it could be possible for a single mAb to be engineered with high affinity for an epitope contained in an oncogene product where the sequence is conserved throughout the malignancy. The protein kinases commonly investigated in targeted cancer therapies come to mind although they might not be suitable candidates because neither the tyrosine, threonine nor serine involved has charged side-chains. However, there appear to be other candidates. For example, c-Myc is considered to be a primary proto-oncogene with one study implicating it in approximately 70 000 cancer deaths per year in the USA (this figure could actually turn out to be a very low estimate given that the data were from 1995 and the number of datasets available for analysis has increased exponentially since then) [8]. In Myc mutations, translocations are common and occur to the immunoglobulin genes on chromosomes 2, 14 and 22 resulting in lymphoid malignancies. Somatic hypermutation occurring after the translocation commonly causes SNPs at Thr58, Ser62 and, significantly, Pro57. These specific mutations have been shown to result in Burkitt's lymphoma. Elevated expression of the c-Myc oncogene has also been reported in lung carcinoma and in one-third of breast and colon carcinomas [6].

If a mAb can be engineered to target an epitope from just this one conserved sequence, widespread applicability could be the result. Works cited here, as well as others not mentioned owing to space constraints, support this notion with one notable example demonstrating that an engineered mAb with specificity for a nucleophosmin 1 mutation showed efficacy in acute myeloid leukemia [9].

The effector: caspase

Caspase-8 was originally chosen for the effector region because of its ability to activate procaspase-3 directly; thus simplifying, it was thought, the regulatory matrix that might inhibit or otherwise negatively affect the signal downstream. Also, it is well studied and starting higher in the pathway seemed preferable because it was thought necessary to produce sufficient signal via the resultant cascade to induce apoptosis. However, after examining the signal