DISTINGUISHED SCIENTIST AWARD

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Targeting the oncogene eIF4E in cancer: From the bench to clinical trials

Abstract

Identifying and targeting specific oncogenes, with the hope that the resultant therapies may eventually prove to exert positive clinical effects, is a major effort in the area of cancer therapeutics. The eukaryotic translation initiation factor, eIF4E, is overexpressed in many cancers, including acute myeloid leukemia. The role of eIF4E in oncogenic transformation and the development of a means to directly target its activity with ribavirin are discussed here. Results from early stage clinical trials and factors contributing to the development of clinical resistance to ribavirin are also described.

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Identifying and targeting specific oncogenes, with the hope that the resultant therapies may eventually prove to exert positive clinical effects, is a major effort in the area of cancer therapeutics. The eukaryotic translation initiation factor, eIF4E, is overexpressed in many cancers, including acute myeloid leukemia. The role of eIF4E in oncogenic transformation and the development of a means to directly target its activity with ribavirin are discussed here. Results from early stage clinical trials and factors contributing to the development of clinical resistance to ribavirin are also described.

The eukaryotic translation initiation factor eIF4E in cancer

One of the main goals in the development of new cancer treatments is to identify the oncogenes that drive cancer formation in cells, and to develop the means of targeting these oncogenes. The eukaryotic translation initiation factor, eIF4E, is a potent oncogene that is found to be dysregulated in approximately 30% of human cancers [1,2]. These cancers include a subset of breast cancers, nearly all head and neck cancers and the M4 and M5 subtypes of acute myeloid leukemia (AML) [1,2]. eIF4E is found in all cell types tested, is evolutionarily conserved and is required for cellular survival [1]. Interestingly, cancer cells characterized by elevated eIF4E are more dependent on eIF4E than normal cells or cancer cells driven by other oncogenes [1]. For instance, suppression of eIF4E with a small molecule inhibitor, such as ribavirin, or by genetic knockdown of eIF4E, preferentially targets the proliferation of cells with elevated eIF4E, relative to cells with normal eIF4E [1,3]. This phenomenon is known as oncogene dependency or oncogene addiction, and is observed for other oncogenes as well.

How does eIF4E drive oncogenesis? eIF4E is found in both the nucleus and the cytoplasm of most cell types [1]. The normal biochemical activity of eIF4E in the cytoplasm is to act in protein synthesis where it actively recruits messenger RNAs (mRNAs) to the ribosome, thereby promoting their translation into protein. In the nucleus, eIF4E acts to transport mRNAs to the cytoplasm, thereby increasing mRNAs availability to the protein synthesis machinery [1]. Importantly, for both cases, eIF4E targets subsets of mRNAs that encode proteins, which, when uncontrolled, may contribute to oncogenic transformation. Thus, highly elevated eIF4E levels preferentially upregulate the production of proteins involved in cell cycle progression, survival and proliferation. In this way, eIF4E can drive oncogenesis. Interestingly, in M4 and M5 AML specimens, eIF4E is both highly elevated and highly enriched in the nucleus, leading to increased mRNA export [1]. One would ex-

pect, depending on the cellular context, a shift in the relative importance of the nuclear and cytoplasmic functions of eIF4E in driving oncogenesis [1].

A critical biochemical feature of eIF4E is its ability to bind a specific moiety on the 5' end of mRNAs known as the 7-methyl guanosine cap (m⁷G cap) [1]. This cap structure is found on all nuclear encoded mRNAs and is absolutely required for the functions of eIF4E in mRNA export and in protein synthesis. There is a specific part of the eIF4E protein that recognizes the m⁷G cap, known as the cap-binding site. Mutation of this cap-binding site, such that it can no longer associate with the m⁷G cap, completely abrogates its activities in mRNA export and protein synthesis and in oncogenic transformation [4]. Furthermore, one cellular protein that strongly represses the functions of eIF4E in transformation, PML, does so by reducing the ability of eIF4E to bind the m⁷G cap by causing structural rearrangements in the eIF4E protein [4,5]. Taken together, targeting the cap binding activity of eIF4E appeared a reasonable strategy for targeting eIF4E dependent cancers.

Targeting eIF4E with ribavirin at the bench and in AML in the clinic

Through serendipitous observations while working with both arenaviruses and eIF4E in my lab in 1997, the idea emerged that the anti-viral drug, ribavirin, could be structurally related to the m⁷G cap and thus, could potentially inhibit eIF4E. After many years of hard work, we established that ribavirin was indeed a competitive inhibitor of the m⁷G cap [3,6,7]. To this end, we used a multitude of biochemical and biophysical studies *in vitro*, including mass spectrometry and nuclear magnetic resonance, to demonstrate that ribavirin bound eIF4E in, or overlapping with, the cap-binding site [3,6,7]. Further, cellular studies indicated that ³H-ribavirin bound eIF4E in living cells [7,8]. Ribavirin treatment targeted the activity of eIF4E both in protein synthesis and in mRNA export and impeded the ability of eIF4E to oncogenically transform cells in culture [3,7-10].

Given our observations that eIF4E was highly elevated in M4 and M5 AML [11], we examined whether ribavirin inhibits colony growth in primary human AML specimens and whether ribavirin targeted the biochemical activity of eIF4E in these specimens [3,7,9]. For comparison, we treated primary M1 and M2 AML specimens with normal eIF4E levels and bone marrow specimens from healthy volunteers. We noted that colony growth was substantially reduced only in the specimens with elevated eIF4E levels [3,7,9]. Ribavirin treatment targeted eIF4E activity in these cells, whereas other oncogenes were driving proliferative and survival gene expression

in the M1 and M2 AML specimens and, thus, ribavirin did not substantially affect these. At these concentrations, ribavirin did not significantly impair the growth of normal bone marrow cells [3,8].

The treatment of AML has not changed significantly since the 1970s, and the standard of care at most institutions still relies on the combination treatment of cytarabine (ara-C) and an anthracycline such as idarubicin or daunorubicin [12,13]. This treatment modality is known as 7+3; referring to the administration of three days of an anthracycline and seven days of ara-C [12,13]. This treatment modality is associated with numerous serious side effects. The median age of AML onset is the late 60s, and, in these patients, the risk of this treatment modality is such that often non-intensive chemotherapy is offered [12,13]. Survival of patients who do not receive intensive chemotherapy is approximately four months [13]. In fact, the National Comprehensive Cancer Network in the US recommend clinical trials as the first option for nearly all AML patients over 60 [12]. For patients 60 and over, the 7+3 treatment regimen leads to a 50% complete remission (CR) frequency but 85% of these patients will relapse, leaving a 10% survival probability at five years from diagnosis [13]. For patients who do achieve a CR with this traditional chemotherapy, remission usually only lasts from two to four months if it is not followed up by consolidation therapy [12].

We examined whether ribavirin could target eIF4E activity in M4 and M5 AML patients and whether this correlated with clinical benefit [14]. This was the first time that eIF4E had been specifically targeted in patients [14]. We monitored the effects of ribavirin monotherapy treatment in patients who had relapsed, were refractory or were unable to undergo standard chemotherapy regimens [14]. This was a multicentre Phase II trial that was run from the Jewish General Hospital in Montreal and also included McMaster Cancer Centre (Hamilton) and Hôpital Maisonneuve Rosemont (Montreal). We observed striking results in this poor prognosis patient population: of 11 evaluable patients, we observed one complete remission, two partial remissions, two blast responses, four stable diseases and two progressive diseases [14]. We saw clear evidence of targeting eIF4E activity in these patients by monitoring mRNA export and levels of target proteins [14]. The most striking observation was that ribavirin treatment, as early as 15 days, caused a massive re-localization of eIF4E from the nucleus to localization in the cytoplasm. Secondary to this re-localization, we often observed large decreases in eIF4E protein and RNA levels. Thus, ribavirin treatment led to the production of cells with normal eIF4E phenotypes. Note that patients who did not respond clinically (i.e., progressive diseases) did not respond

molecularly (i.e., their eIF4E activity and levels were not targeted). Patient responses were, unfortunately, transitory with average responses in the approximately two to four month range and all patients who responded eventually became both clinically and molecularly resistant to ribavirin. The patient who achieved a complete remission was on protocol for nine months. The appearance of resistance was not a surprise, as most targeted monotherapies lead to resistance in this same time frame, this is true even for trans-retinoic acid treatment of acute promyelocytic leukemia, where this treatment must be followed by consolidation therapy in order to obtain a durable outcome [15].

We are clearly excited by the responses that we observed, but are determined to develop modalities that improve both frequency and durability of response. As alluded to above for APL, durable outcomes are frequently achieved by combining the targeted monotherapy with a traditional cytotoxic chemotherapy. Thus, we are currently exploring the utility of treating this same patient population with ribavirin and low dose ara-C (Clinical Trials.gov NTC01056523); a protocol which has recently been used for the treatment of elderly patients who cannot tolerate high dose ara-C. In a Phase I clinical trial with the same multi-centre team, we have observed positive clinical responses in this patient population, including remissions and blast responses, but the Phase I study is ongoing and thus we will have to wait for the Phase II portion in order to determine the utility of this combination.

Ribavirin resistance: impact on patient selection and drug combination strategies

Given our monotherapy results, we also wanted to better understand the molecular basis of ribavirin resistance so that we can overcome, or perhaps even prevent, the onset of resistance. To this end, we developed ribavirin-resistant cell lines in the lab and identified two classes of drug resistance [16]. In one form, ribavirin uptake is severely impaired. In some cases this is due to modulation of a nucleoside transporter, which is used by both ribavirin and ara-C [16]. In the other form of resistance, ribavirin uptake is normal but the ribavirin-eIF4E interaction is lost. We have started to dissect the molecular mechanisms that underlie this resistance and have developed means to pharmacologically modulate resistance in the latter type. The clinical utility of this strategy is certainly a future goal of this work.

Hopes and Challenges for the Future.

Although we are excited about the striking initial positive clinical observations in poor prognosis AML patients, we have

many challenges ahead. First and foremost is to develop effective combination therapies to prevent resistance and thus establish durable outcomes in these patients. Given that ribavirin resistance may occur due to treatment with other chemotherapies, such as ara-C, we hope to eventually have the opportunity to determine the efficacy of ribavirin on less heavily pretreated patient populations. Second, we would like to establish the efficacy of targeting eIF4E in other malignancies and, to this end, our collaborator, Dr. Wilson Miller, has embarked on a phase I trial in solid tumour patients (Clinical Trials.gov NCT01056757). It may be that all eIF4E high cancers will not be equally sensitive to ribavirin; thus, finding the best context for this treatment is critical. Understanding which genes cooperate with eIF4E will help us to develop better targeting strategies. It is important to note that, like all small molecules, ribavirin undoubtedly has off-target effects [7]. As we understand its effects better, we will be able to assess if these off-target effects are beneficial or not.

Ribavirin, as a generic, is one of many potentially valuable old drugs. The first reports on the antiviral activities of ribavirin were made in 1972 [17], leaving very little intellectual property space. This status has helped our studies, as there is a ready supply of ribavirin to try in the lab from many commercial suppliers and we started with substantial knowledge of the pharmacology of the drug allowing us to embark on a Phase II trial straight away. The drug's status has also hindered the development of ribavirin as the traditional big pharma funding mechanisms for such studies were not available to us. In fact, we have been advised on multiple occasions that we need to make chemically distinct forms of ribavirin, not to achieve better activity, which would have been an excellent reason for doing so, but rather in order to establish a firm intellectual property position. Instead of finding a new ribavirin (or other generic drugs), perhaps the medical community collectively needs to rethink our drug development strategies. The support for the clinical trials is from the Leukemia and Lymphoma Society (USA). Without their support, none of this work would be possible and I can never thank them enough.

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