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Have we made progress in the treatment of GVHD?

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One reason for the lack of progress in the treatment of acute graft versus host disease (GVHD) is the lack of reliable biomarkers. GVHD of the gastrointestinal (GI) tract is closely associated with non-relapse mortality (NRM) following hematopoietic cell transplantation (HCT). Using an unbiased, large-scale, quantitative proteomic discovery approach, we identified candidate biomarkers that were increased in plasma from HCT patients with GI GVHD. We then validated the lead candidate, REG3α, by ELISA in samples from more than 1000 HCT patients from three transplant centers. Plasma REG3α concentrations were 3-fold higher in patients at GI GVHD onset than in all other patients. REG3α concentrations correlated most closely with lower GI GVHD at GVHD onset and predicted response to therapy at 4 weeks, 1-year NRM, and 1-year survival ($P \leq 0.001$). Multivariate analysis showed that advanced clinical stage, severe histologic damage, and high REG3α concentrations at the diagnosis of GVHD independently predicted 1-year NRM, which progressively increased with higher numbers of onset risk factors present. We conclude that REG3α is a plasma biomarker of GI GVHD that can be combined with clinical stage and histologic grade to improve risk stratification of patients, perhaps providing a platform for advances in the treatment of high-risk GVHD.

Introduction

Allogeneic hematopoietic cell transplantation (HCT) is one of the best curative modalities for patients with intermediate- and high-risk acute leukemia; approximately 3,500 patients receive allo-HCT for acute leukemia annually [1]. The efficacy of this therapy is limited by the development of acute graft versus host disease (GVHD), which is measured by dysfunction in three organ systems: the skin,

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liver and gastrointestinal (GI) tract [2,3]. Acute GVHD of the GI tract affects up to 60% of patients receiving allogeneic HCT [4,5], causing nausea, vomiting, anorexia, secretory diarrhea and, in more severe cases, severe abdominal pain and/or hemorrhage [6]. Acute GVHD is often clinically indistinguishable from other causes of GI dysfunction such as conditioning regimen toxicity, infection, or medication effect. Endoscopic biopsy is often used to confirm the diagnosis [7], but histologic severity on biopsy does not consistently correlate with clinical outcome [2,7,8]. Clinical stage two or greater (more than 1 L of diarrhea per day) is associated with reduced survival [4,5], but daily stool volume can vary considerably. Lower GI GVHD responds poorly to treatment compared to other target organs [5], and treatment with high-dose systemic steroid therapy carries significant risks, especially infectious complications in profoundly immunosuppressed patients [9,10]. The standard treatment of acute GVHD is high dose systemic steroids, which has not changed in 40 years. One reason for this lack of progress is the lack of validated biomarkers for acute GVHD. We have recently identified and validated regenerating islet-derived 3-alpha (REG3α), a C-type lectin secreted by Paneth cells [11,12], as a noninvasive, reliable blood biomarker specific for GVHD of the GI tract with diagnostic and prognostic utility that may provide a platform for novel advancements in the treatment of GVHD [13] (Table 1).

Discovery proteomics

We used the Intact Protein Analysis System proteomics approach to identify candidate biomarkers in a discovery set of pooled plasma samples taken at similar times after HCT from 10 patients with biopsy-proven GI GVHD and 10 patients without GVHD as previously described [14,15]. We identified and quantified 562 proteins of which 74 were increased at least two-fold in patients with GVHD. Of the 5 preferentially expressed in the GI tract, commercially available antibodies suitable for quantification of plasma concentrations by ELISA were available for only 1 of these 5 proteins, thus identifying Regenerating Islet-Derived 3-Alpha as our lead candidate (Fig. 1).

Validation studies

We evaluated REG3α plasma concentration as a biomarker of GI GVHD in samples from a large validation set of allogeneic HCT recipients from the University of Michigan. Plasma REG3α concentrations were 3 times higher in patients at the onset of GI GVHD than in all other patients, including those with non-GVHD enteritis (Fig. 2A). There was no specific cause of non-GVHD enteritis associated with higher REG3α concentrations (data not shown). Serum REG3α concentrations were also higher in GI GVHD in an independent validation set of 143 HCT patients from Regensburg, Germany, and Kyushu, Japan, although the absolute values were lower (Fig. 2B). This difference may be due to a center effect that depends on several factors, including variations in transplant conditioning regimens and supportive care. For example, all patients in Regensburg and Kyushu received oral antibiotics as GVHD prophylaxis, whereas Michigan patients did not and thus increased GI flora might account for greater REG3α secretion.

Table 1
One year NRM at the onset of lower GI GVHD based upon number of high-risk factors present at onset (REG3α concentration, clinical stage, histologic severity.

Number of risk factors	1-year NRM	*p*-value*
0	25%	
1	34%	0.2[a]
2	66%	<0.001[b]
3	86%	<0.001[c]

**P*-values adjusted for donor source, HLA match, conditioning intensity, recipient age, and baseline disease severity according to the Center for International Blood and Marrow Transplant Research (CIBMTR) guidelines.

[a] 0 vs. 1 risk factor.

[b] 1 vs. 2 risk factors.

[c] 2 vs. 3 risk factors.