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Regeneration-associated WNT Signaling Is Activated in Long-term Reconstituting AC133^{bright} Acute Myeloid Leukemia Cells[1,2]

Alessandro Beghini*, Francesca Corlazzoli*,[3], Luca Del Giacco†,[3], Matteo Re‡, Francesca Lazzaroni*, Matteo Brioschi§, Giorgio Valentini‡, Fulvia Ferrazzi¶,#, Anna Ghilardi†, Marco Righi*,, Mauro Turrini††, Marco Mignardi‡‡,§§, Clara Cesana††, Vincenzo Bronte¶¶, Mats Nilsson‡‡,§§, Enrica Morra†† and Roberto Cairoli††,##**

*Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, Milano, Italy; †Department of Biosciences, Università degli Studi di Milano, Milano, Italy; ‡Department of Computer Science, Università degli Studi di Milano, Milano, Italy; §Dipartimento di Scienze Oncologiche e Chirurgiche, Università degli Studi di Padova, Padova, Italy; ¶Dipartimento di Informatica e Sistemistica, Università degli Studi di Pavia, Pavia, Italy; #Gene Center, Ludwig-Maximilians-Universität München, Munich, Germany; **Consiglio Nazionale delle Ricerche, Institute of Neuroscience, Milano, Italy; ††Department of Oncology, Niguarda Hospital, Milano, Italy; ‡‡Department of Immunology, Genetics and Pathology, Rudbeck Laboratory, Uppsala Universitet, Uppsala, Sweden; §§Science for Life Laboratory, Department of Biochemistry and Biophysics, Stockholm University, Solna, Sweden; ¶¶Istituto Oncologico Veneto, Padova, Italy; ##Divisione di Medicina Interna, Sezione di Ematologia Clinica, Ospedale Valduce, Como, Italy

Abstract

Acute myeloid leukemia (AML) is a genetically heterogeneous clonal disorder characterized by two molecularly distinct self-renewing leukemic stem cell (LSC) populations most closely related to normal progenitors and organized as a hierarchy. A requirement for WNT/β-catenin signaling in the pathogenesis of AML has recently been suggested by a mouse model. However, its relationship to a specific molecular function promoting retention of self-renewing leukemia-initiating cells (LICs) in human remains elusive. To identify transcriptional programs involved in the maintenance of a self-renewing state in LICs, we performed the expression profiling in normal (n = 10) and leukemic (n = 33) human long-term reconstituting AC133^{+} cells, which represent an expanded cell population in most AML patients. This study reveals the ligand-dependent WNT pathway activation in AC133^{bright} AML cells and shows a diffuse

Abbreviations: AML, acute myeloid leukemia; LSC, leukemic stem cell; LT-HSCs, long-term hematopoietic stem cells; CFU, colony forming units; CFU-GM, granulocyte/macrophage; BM MNCs, bone marrow mononuclear cells; LIC, leukemia-initiating cell

Address all correspondence to: Alessandro Beghini, PhD, Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, via Viotti 3/5, 20133 Milano, Italy. E-mail: alessandro.beghini@unimi.it

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[3]F. Corlazzoli and L. Del Giacco contributed equally to this work.
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expression and release of WNT10B, a hematopoietic stem cell regenerative-associated molecule. The establishment of a primary AC133$^+$ AML cell culture (A46) demonstrated that leukemia cells synthesize and secrete WNT ligands, increasing the levels of dephosphorylated β-catenin *in vivo*. We tested the LSC functional activity in AC133$^+$ cells and found significant levels of engraftment upon transplantation of A46 cells into irradiated Rag2$^{-/-}\gamma c^{-/-}$ mice. Owing to the link between hematopoietic regeneration and developmental signaling, we transplanted A46 cells into developing zebrafish. This system revealed the formation of ectopic structures by activating dorsal organizer markers that act downstream of the WNT pathway. In conclusion, our findings suggest that AC133^{bright} LSCs are promoted by misappropriating homeostatic WNT programs that control hematopoietic regeneration.

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Introduction

Different genetic causes result in variable clinical courses of acute myeloid leukemia (AML) and different responses to standard chemotherapy including stem cell transplant. Despite the genetic differences among individual patients, most AML clones display certain common features. Ample evidence exists in mouse models that AML develops through the stepwise acquisition of collaborating genetic and epigenetic changes in self-renewing LICs, which exhibit a committed myeloid immunophenotype and give rise to nonleukemogenic progeny in a myeloid-restricted hierarchy [1–3]. An important issue to understand the early events in the origin of AML is the observation that long-term hematopoietic stem cell (LT-HSC) expansion precedes the generation of committed myeloid LICs [4].

Although well-orchestrated cell intrinsic programs and environmental cues represent the main contributory factors for normal LT-HSC expansion, it is still unclear if transcriptional programs responsible for the expansion of premalignant LT-HSC populations and leukemia initiation share common embryonic or post-embryonic functions, such as stem cell renewal, tissue repair, and regeneration [5,6]. Even though recent studies have addressed the role of Hedgehog signaling for maintenance of cancer stem cells in myeloid leukemia [7], its requirement in AML remains controversial [8].

Recently, the notion that LICs are restricted only to the CD34$^+$CD38$^-$ population has been challenged [9,10] and it has been suggested that more cell surface markers could be appropriately used to enrich the leukemia-initiating cell (LIC)–containing fraction. One of such markers is the AC133 antigen (a glycosylation-dependent epitope of CD133) that defines a desirable population of stem and progenitor cells containing in turn all the CD34^{bright}CD38$^-$ progenitors, as well as the CD34^{bright}CD38$^+$ cells committed to the granulocytic/monocytic lineage [11]. In addition, AC133 represents a well-documented marker of tumor-initiating cells in a number of human cancers [12]. In this study, fluorescence-activated cell sorter (FACS) analysis demonstrates that AC133$^+$ cell population is dramatically expanded in 25 AML cases analyzed. We carried out genome-wide transcriptional analysis of AC133$^+$ cells isolated from newly diagnosed non-promyelocytic AML patients (n = 33) and healthy donors (n = 10). Results obtained from a multistep analysis of the generated data defined the involvement of the ligand-dependent WNT receptor signaling pathway as the self-renewal associated signature in the AC133-enriched fraction in human AML. Furthermore, the results presented here suggested that *WNT10B* and other WNT genes expressed during the regenerative process of the hematopoietic system [13,14] are aberrantly upregulated in AC133^{bright} AML cells. To obtain a localized detection of each single transcript, we first applied an *in situ* detection of individual mRNA molecules [15] on bone marrow (BM) sections from AML patients. By the establishment of a primary culture of AC133$^+$ AML cells (termed A46 hereafter), we confirmed that secreted WNTs activated a β-catenin/human T-cell factor (TCF) transcription–based reporter construct. Moreover, we intend to clarify the relationship between the abnormal WNT activation in AC133$^+$ population and the leukemic stem cell (LSC) activity. Using Rag2$^{-/-}\gamma c^{-/-}$ as immunodeficient xenotransplant model [16], AC133$^+$ A46 cells were injected intravenously into sublethally irradiated mice.

To achieve a complete view of how AC133$^+$ A46 cells modulated the microenvironment and given that hematopoietic regeneration converge to developmental signaling, we used zebrafish embryonic model as an *in vivo* biosensor.

Our results confirmed previously reported data [17] and raise new important implications for the involvement of the ligand-dependent canonical WNT pathway in AML. These suggestive findings are supported by the pivotal function of WNT in promoting self-renewal [18,19], its emerging role in myeloid leukemogenesis [20,21], and the effects of its constitutive activation through a stabilized form of β-catenin, by inducing quiescent stem cells to enter the cell cycle and arresting their differentiation [22,23].

Materials and Methods

Collection of Patient Samples and Normal Hematopoietic Cells

BM MNCs were collected from 33 newly diagnosed, unselected non-promyelocytic AML patients, according to Niguarda Hospital's Ethical Board–approved protocols (116_04/2010). According to the revised Medical Research Council risk group stratification, based on cytogenetic and molecular markers/mutations [24], samples included 14 adverse, 13 intermediate, and 6 favorable risk patients. Human adult BM cells obtained from 10 consenting healthy donors were processed as previously described [25].

Cell Sorting and Flow Cytometry

We carried out AC133$^+$ cell separation based on MACS MicroBeads and cytofluorometric determinations, as previously described [25].

Microarray Expression Analysis

Total RNA for expression profiling was extracted using RNAqueous-4PCR kit (Ambion, Austin, TX) from AC133-selected cells. Expression profiling was performed on Affymetrix HGU133plus2.0 GeneChip