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Biochemical mechanisms implemented by human acute myeloid leukaemia cells to suppress host immune surveillance

Inna M. Yasinska[1], Isabel Gonçalves Silva[1], Svetlana Sakhnevych[1], Bernhard F. Gibbs[2], Ulrike Raap[2], Elizaveta Fasler-Kan[3,4], Vadim V. Sumbayev[1]*

1 – Medway School of Pharmacy, Universities of Kent and Greenwich, Chatham Maritime, United Kingdom

2 - Department of Dermatology and Allergy, University of Oldenburg, Germany

3 - Department of Pediatric Surgery and Department of Clinical Research, Children's Hospital, Inselspital, University of Bern, Switzerland

4 – Department of Biomedicine University Hospital Basel and University of Basel, Switzerland

* **- To whom correspondence should be addressed:** Vadim V. Sumbayev:

V.Sumbayev@kent.ac.uk

Human malignant tumours have developed a variety of effective molecular strategies which allow them to escape host immune surveillance leading to a disease progression. This includes haematological malignancies such as acute myeloid leukaemia (AML), a blood/bone marrow cancer originating from self-renewing myeloid cell precursors which rapidly becomes systemic. AML cells are capable of escaping immune attack despite being permanently exposed to host immune cells including cytotoxic T cells (CTCs) and natural killer (NK) cells[1]. AML cells successfully implement biochemical mechanisms which allow them to inactivate cytotoxic lymphoid cells (NK cells and CTCs) upon direct contact as well as at a distance[2]. In this case they not only "fight back" against immune cells but also effectively prevent the actual process of cytotoxic immune attack. In this work we will discuss several important biochemical mechanisms which allow AML cells to form immunological synapses with cytotoxic lymphoid cells and also comprehensively inactivate anti-cancer immunity at a distance.

T helper (Th) type cells generate and secrete interleukin-2 (IL-2), a stimulatory cytokine which triggers activation of NK cells as well as CTCs[3]. Upon activation, these cytotoxic lymphoid cells become capable of attacking malignant (AML for example) cells delivering the proteolytic enzyme granzyme B into them. Granzyme B itself can directly activate one of the key apoptotic enzymes, caspase-3. However, granzyme B performs cleavage of the pro-apoptotic protein Bid, forming its active form tBid which negatively impacts on mitochondrial function, inducing release of cytochrome c, one of the major components of electron respiratory chain. Cytochrome c interacts with apoptotic protease activating factor-1 (Apaf-1) and pro-caspase-9 thus forming an apoptosome, which induces programmed death of target cell[2].

It has become evident that AML cells are capable of expressing surface proteins such as programmed death-1 (PD-1) receptor ligands (PD-Ls) 1 and 2 as well as CD86, the ligand of

48   cytotoxic T-cell antigen 4 (CTLA4)[1]. T helpers and CTCs/NK cells express PD-1 receptors

49   on their surface. AML cell surface-based PD-1 ligands 1 and 2 (PD-L(i)) interact with PD-1

50   on lymphoid cell surfaces. As a result, T helper cells stop producing IL-2 required for the

51   activation of both CTCs and NK cells. PD-1 signalling attenuates the protein kinase C θ

52   (PKC-θ) loop thus preventing activation of transcription factors NF-κB and AP-1, which are

53   required for IL-2 production[4].

54   On the other hand, the interaction of PD-Ls with PD-1 on the surface of NK cells and CTCs

55   leads to their rapid inactivation, and, as a result, they lose ability to kill AML cells[1]. In

56   addition, AML cells are often capable of expressing the CTLA4 ligand CD86. Interaction of

57   CD86 with CTLA4 rapidly leads to inactivation of effector T cells[1,5].

58   Thus, one could conclude that CD86 and PD-Ls 1 and 2 are involved in the formation of

59   immunological synapses with both regulatory and cytotoxic lymphoid cells leading to

60   downregulation of the biochemical activation of CTCs and NK cells. Direct interaction of

61   PD-Ls and CD86 with CTCs and NK cells leads to loss of their anti-cancer activities.

62   Schematically this process is shown in Figure 1.

63   Recent evidence also demonstrated the ability of AML cells to downregulate the activity of

64   cytotoxic lymphoid cells through lymphocyte-activation gene 3 (LAG-3), which is a homolog

65   of CD84. AML cells were reported to induce exhaustion of cytotoxic lymphoid cells through

66   LAG-3 but detailed mechanisms of this event remain to be elucidated[1,5].

67   Recently, it has become evident that the immune receptor Tim-3 (T cell immunoglobulin and

68   mucin domain containing protein 3) is involved in protecting AML cells against host immune

69   surveillance[2,6]. Tim-3 has a natural ligand galectin-9 (a tandem protein which contains two

70   receptor-binding domains fused together by a peptide linker) which was suggested to form an

71   autocrine loop with the receptor[7]. When present on the cell surface, galectin-9 induces Tim-3

72   downstream signalling which includes activation of pathways responsible for cell survival[7-9].

73   This first of all includes activation of transcription factor nuclear factor kappa B (NF-kB)[7],

74   translational pathways controlled by mammalian target of rapamycin (mTOR) and hypoxic

75   signalling required for the adaptation of AML cells to stress conditions and their survival in

76   general[8,9]. The Tim-3-galectin-9 complex was also reported to activate the β-catenin pathway

77   which, together with NF-kB, controls self-renewal of AML cells[7]. Taken together, one may

78   conclude that galectin-9 mediates survival signalling through Tim-3 (Figure 2).

79   Galectin-9 lacks a secretory domain and thus requires a trafficker in order to be taken to the

80   cell surface and then secreted[2,10]. We have recently found that AML cells but not healthy

81   leukocytes express the neuronal receptor latrophilin 1 (LPHN1). LPHN1 is expressed in

82   haematopoietic stem cells, but disappears upon maturation unless they undergo malignant

83   transformation into AML cells. Using its natural ligands (for example fibronectin leucine rich

84   transmembrane protein 3, FLRT3), LPHN1 facilitates exocytosis of Tim-3-galectin-9, which

85   then triggers cell survival signalling. However, Tim-3 either on its own or in complex with

86   galectin-9 can also be proteolytically shed from the surface of AML cells thus leading to

87   secretion of both proteins. Galectin-9 interacts with NK cells and CTCs (most likely though

88   Tim-3)[2]. This leads to impairing of cytotoxic activity of NK cells and killing of CTCs.

89   Interestingly, NK cells produce interferon gamma (IFN-γ) in response to stimulation with

90   galectin-9. IFN-γ induces the activation of indoleamine 2,3-dioxygenase (IDO1), an enzyme

91   converting L-tryptophan into formyl-L-kynurenine, which is then degraded into L-kynurenine

92   and released[12]. L-kynurenine impairs the cytotoxic activity of NK cells[2].

93   Intriguingly, IFN-γ is also known to induce the expression of PD-Ls [13], which might further

94   promote the ability of AML cells to protect themselves against host immune surveillance.

95   Soluble Tim-3 released by AML cells is capable of downregulating IL-2 secretion by Th cells

96   acting via a receptor which remains to be identified[2]. This prevents the activation of cytotoxic

97   lymphoid cells. Importantly, the secretion of Tim-3 and galectin-9 allows AML cells to

98   supress cytotoxic lymphoid cells at a distance thus minimising direct interaction with them.

99   This allows AML cells to "focus on" self-renewal thus leading to rapid disease progression.

100   The functioning of the Tim-3-galectin-9 secretory and signalling pathway in AML cells is

101   summarised in Figure 2.

102   Importantly, stress associated with the events described above leads to release of high

103   mobility group box 1 (HMGB1) protein by AML cells which finally triggers production of

104   interleukin 1 beta (IL-1β) by healthy leukocytes[14]. IL-1β was reported to induce the

105   expression and production of stem cell factor (SCF) by epithelial cells via mTOR pathway

106   and hypoxic signalling[15]. SCF is a major hematopoietic growth factor that controls the AML

107   progression thus becoming highly oncogenic[15]. In such a way, AML cells employ body

108   systems to produce factors required for their proliferation/disease progression[14,15].

109

110   Taken together, it is clear that AML cells implement comprehensive mechanisms in order to

111   escape immune surveillance and progress the disease. Pharmacological targeting of the

112   biochemical pathways responsible for immune escape will enable the human immune system

113   to potentially cure AML and thus avoid aggressive chemotherapy and bone marrow

114   transplantation. Therefore, design and development of new strategies for anti-AML

115   immunotherapy are a major focus for current applied AML research. It is also vital to

116   investigate whether other cancers operate similar mechanisms since certain solid tumours

117   (e.g. colon cancer[16]) were already reported to use the Tim-3-galectin-9 loop for immune

118   evasion.

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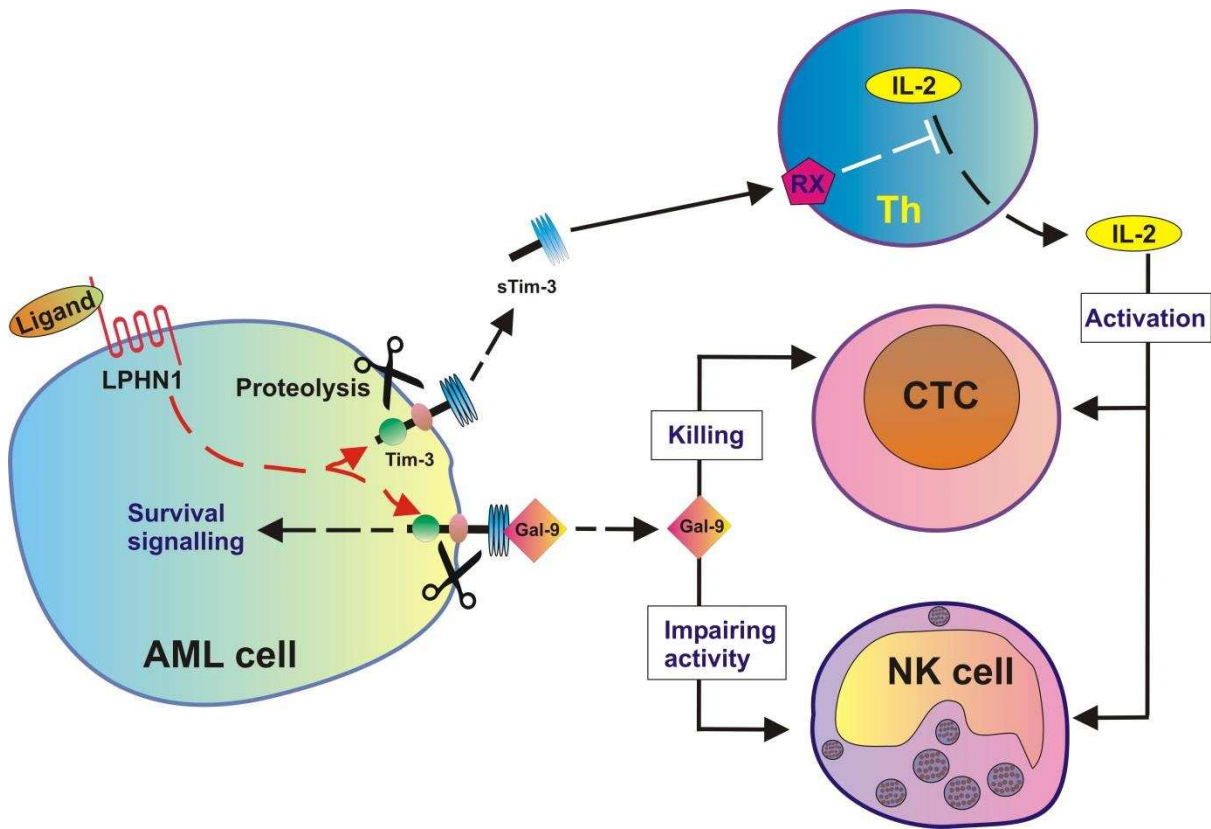171   2015, **5:** 15659.

172 **Figure Legends**

173 **Figure 1. AML cells supress IL-2 production and the activity of cytotoxic lymphoid cells**

174 **via PD-1 and CTLA4 receptors**
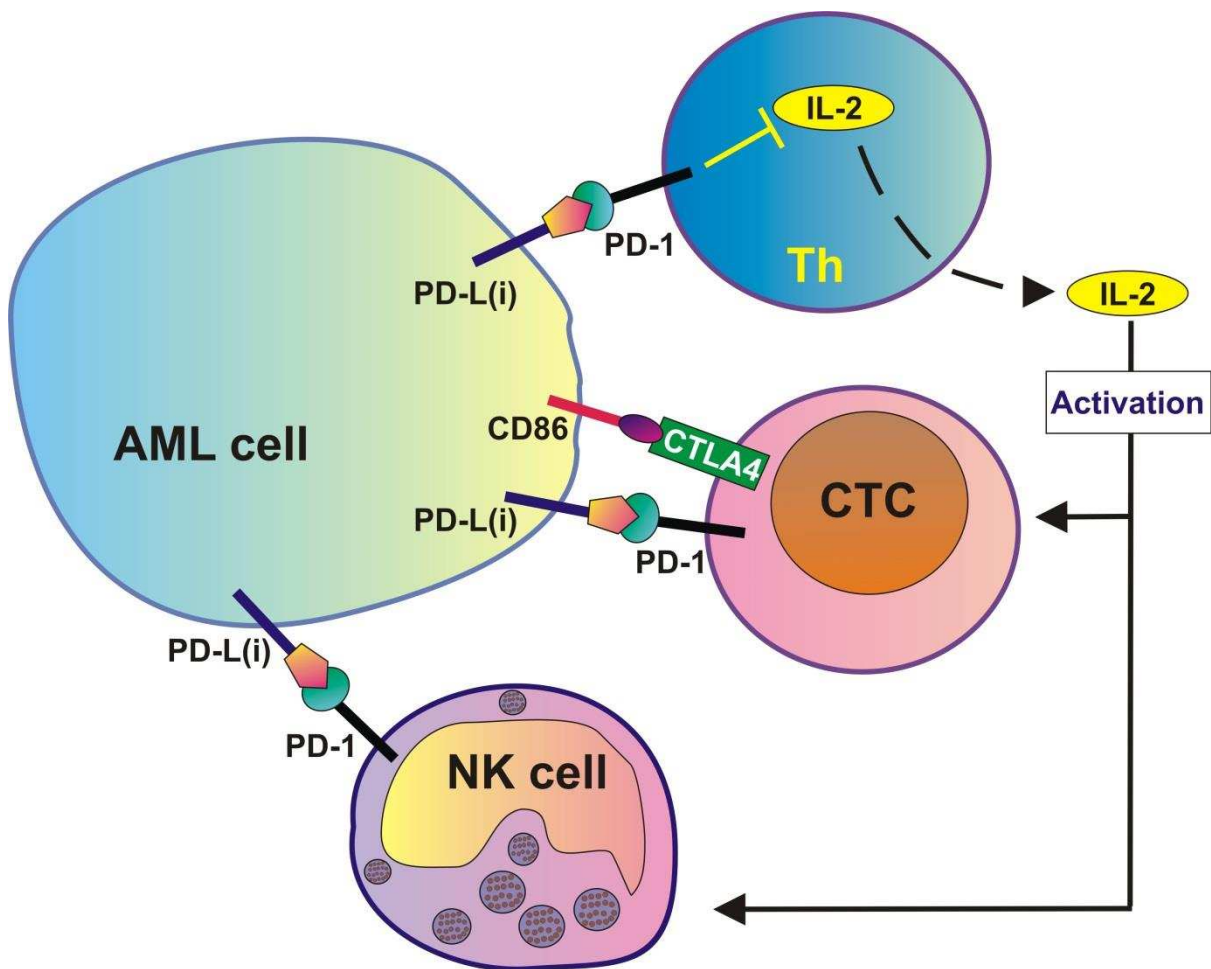
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177 **Figure 2. The Tim-3-galectin-9 pathway regulates both intracellular AML cell survival**

178 **signalling and immune escape.**

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