

**DIFFERENTIAL DNA METHYLATION OF NK CELLS PREVENTS
THEIR EXCESSIVE ACTIVATION**

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ПРЕДОТВРАЩАЕТ ЧРЕЗМЕРНУЮ АКТИВАЦИЮ НК-КЛЕТОК

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Abstract

Background and Objectives: Epigenetic mechanisms, particularly DNA methylation, represent a key regulatory layer governing immune cell differentiation, lineage commitment, and functional adaptation. Progressive alterations in DNA methylation accompany immune cell maturation and contribute to the emergence of specialized immune subsets. Although several lineage-specific methylation signatures have been identified, the broader role of DNA methylation in regulating immune activation remains incompletely defined. This study aimed to summarize current evidence on how DNA methylation shapes immune cell identity and modulates activation dynamics, with particular emphasis on regulatory T cells and natural killer (NK) cells.

Methods: Evidence from recent epigenomic and immunological studies was examined to characterize methylation patterns associated with immune cell differentiation and activation. Particular attention was given to studies identifying differentially methylated regions in key regulatory loci and genes involved in immune signaling pathways.

Results: DNA methylation patterns were found to correlate strongly with immune cell lineage specification and functional states. Demethylation of the FOXP3 locus represents a hallmark epigenetic signature of CD4⁺ regulatory T cells and is essential for their stable differentiation and maintenance of immune tolerance. Similarly, reduced methylation at CpG sites within the NCR1/NKp46 locus is significantly associated with NK cell abundance and spatial distribution. In activated NK cells, multiple differentially methylated regions have been identified in genes involved in inflammatory regulation. Notably, altered methylation states of IL1RN, ECE1, CSF2, and DCHS1 were significantly associated with transcriptional repression during activation (reported $p < 0.05$ in the referenced studies), suggesting a regulatory mechanism that constrains excessive inflammatory responses.

Conclusion: DNA methylation plays a central role in orchestrating immune cell differentiation and modulating activation responses. Specific methylation

signatures, such as those observed in FOXP3 and NCR1, serve as robust biomarkers of immune cell identity, while dynamic methylation changes in genes including IL1RN, ECE1, CSF2, and DCHS1 contribute to the fine-tuning of NK cell activity. A deeper understanding of these epigenetic mechanisms may support the development of targeted therapeutic strategies aimed at modulating immune responses while preserving immune homeostasis.

Keywords: NK cells, Immune system, DNA methylation, Immunology, Epigenetics, Genes.

Резюме

Введение и цели: Эпигенетические механизмы такие как метилирование ДНК, представляют собой ключевой фактор регуляции, управляющий дифференцировкой иммунных клеток, определением дифференцировки клеточной линии и функциональной адаптацией. Изменения в метилировании ДНК сопровождают созревание иммунных клеток и способствуют появлению специализированных иммунных субпопуляций. Несмотря на описание ряда специфических для клеточных линий маркеров метилирования, более широкое значение метилирования ДНК в регуляции активации иммунной системы остается изученной недостаточно. Целью настоящего исследования было обобщение имеющихся данных о роли метилирования ДНК на идентичность иммунных клеток и модулирования динамики активации, прежде всего для регуляторных Т-клеток и естественных клеток-киллеров (НК-клетки).

Методы: Были изучены данные недавних эпигеномных и иммунологических исследований по характеристике паттернов метилирования, связанных с дифференцировкой и активацией иммунных клеток. Особое внимание было уделено исследованиям, выявившим дифференциально метилированные регионы в ключевых регуляторных локусах и генах, участвующих в сигнальных путях иммунной системы.

Результаты: Было обнаружено, что паттерны метилирования ДНК сильно коррелируют со спецификацией формированием иммунных клеток и их функциональным состоянием. Деметилирование локуса FOXP3 представляет собой характерную эпигенетическую сигнатуру регуляторных CD4⁺ Т-клеток и имеет важное значение для их стабильной дифференцировки и поддержания иммунной толерантности. Аналогично, снижение метилирования в CpG-сайтах в локусе NCR1/NKp46 существенно ассоциировано с количеством НК-клеток и пространственным

распределением. В активированных НК-клетках были выявлены множественные дифференциально метилированные регионы в генах, участвующих в регуляции воспаления. Примечательно, что изменение состояния метилирования генов IL1RN, ECE1, CSF2 и DCCHS1 были достоверны связаны с подавлением транскрипции во время активации ($p < 0,05$ в цитируемых исследованиях), что предполагает наличие регуляторного механизма, ограничивающего чрезмерные воспалительные реакции.

Заключение: Метилирование ДНК играет центральную роль в организации дифференцировки иммунных клеток и модулировании реакций активации. Специфические маркеры метилирования, отмеченные в генах FOXP3 и NCR1, служат надежными биомаркерами идентичности иммунных клеток, в то время как динамические изменения метилирования в генах, включая IL1RN, ECE1, CSF2 и DCCHS1, способствуют тонкой настройке активности НК-клеток. Более глубокое понимание указанных эпигенетических механизмов может способствовать разработке прицельных терапевтических стратегий по модулированию иммунных ответов при сохранении иммунного гомеостаза.

Ключевые слова: НК-клетки, Иммунная система, Метилирование ДНК, Иммунология, Эпигенетика, Гены.

1 **1 Introduction**

2 Epigenetic regulatory systems—most prominently DNA methylation—
3 represent a foundational layer of biological control that dictates how the immune
4 system establishes, maintains, and refines lineage-specific differentiation patterns
5 [1-6]. As multipotent hematopoietic progenitors progress through successive
6 developmental stages, their initially flexible epigenetic architecture undergoes
7 progressive restriction, resulting in the stabilization of distinct immunological
8 identities. This narrowing of developmental potential is not merely a passive
9 outcome of differentiation but rather an actively guided process in which DNA
10 methylation operates as a chemically robust and mitotically heritable mark that
11 contributes both to the initiation and reinforcement of stable cell fate trajectories [4].
12 Consequently, understanding how methylation patterns are written, erased, and
13 interpreted has become central to the broader effort to decode the molecular logic of
14 immune system development. A wealth of methylome-wide studies has interrogated
15 the epigenetic transitions that accompany differentiation along major hematopoietic
16 lineages, including erythropoietic, T-lymphocytic, B-lymphocytic, and myeloid
17 pathways [29, 11, 12, 13, 16]. These investigations have illuminated common
18 principles, such as the selective gain or loss of methylation at key transcriptional
19 regulators, as well as lineage-specific blueprints that define the molecular
20 boundaries between one immune subset and another. Beyond revealing the
21 architecture of immune development, these studies provide an expanding repertoire
22 of epigenetic biomarkers capable of discriminating immune cell states with far
23 greater precision than transcriptional profiles alone. Such markers have proven
24 especially valuable in contexts where transcriptional signatures are transient or
25 reversible, whereas methylation signatures offer durable and historically informative
26 insights into a cell's developmental origin. A canonical example of an epigenetic
27 signature that tracks with immune lineage identity is the demethylated state of the
28 FOXP3 locus, which serves as a defining molecular hallmark of CD4⁺ regulatory T
29 (Treg) cells [27]. The stability of FOXP3 demethylation ensures continued

30 expression of this transcription factor, which functions as the master regulator of
31 Treg development, suppressive capacity, and long-term lineage fidelity. A
32 comparable paradigm exists within the natural killer (NK) cell compartment:
33 hypomethylated CpG regions within the NCR1/NKp46 gene locus correlate strongly
34 with NK cell abundance, tissue distribution, and activation potential, thereby serving
35 as both a developmental signature and a functional predictor [1]. These examples
36 illustrate how targeted methylation patterns can encode cell identity in a manner that
37 remains resilient across cell divisions and environmental perturbations. Despite
38 these notable advances, our understanding of how DNA methylation contributes to
39 the rapid and often transient changes associated with immune activation remains
40 incomplete. Transcriptomic analyses have consistently shown that pathogen
41 exposure, cytokine stimulation, and intercellular signaling events initiate extensive
42 waves of gene expression remodeling across a broad array of immune cell types.
43 However, the role of DNA methylation—whether as a driver, stabilizer, or
44 consequence of these activation-induced transcriptional changes—remains only
45 partially elucidated [2, 3, 7, 10, 19]. Activation, unlike differentiation, is a highly
46 dynamic process; thus, the temporal coordination between methylation changes and
47 functional outputs has proven difficult to delineate fully. Nevertheless, several
48 activation-associated loci have been examined in considerable detail. For example,
49 stimulation with interleukin-2 (IL-2) or engagement of the T cell receptor (TCR)
50 induces targeted demethylation of the IFNG locus in CD4⁺ Th1 cells, thereby
51 enhancing their ability to secrete interferon- γ (IFNG), a cytokine central to antiviral
52 immunity and macrophage activation [21, 5]. Similarly, studies of CD8⁺ T cells have
53 revealed substantial epigenetic fluidity at the CD8A co-receptor locus during
54 memory formation, with methylation states fluctuating according to cytokine
55 exposure and polarization conditions such as Th1- or Th2-skewed milieus [9].
56 Complementing these observations, genome-wide CpG mapping of CD4 helper T
57 cells has demonstrated that activation triggers an extensive reconfiguration of both
58 global and locus-specific methylation landscapes, suggesting that epigenetic

59 plasticity is an intrinsic feature of T cell responsiveness rather than a rare
60 phenomenon restricted to development [17]. Within the myeloid compartment,
61 analogous methylation changes occur during the differentiation of human CD14⁺
62 monocytes into immature and mature dendritic cells, underscoring the widespread
63 relevance of epigenetic remodeling to both innate and adaptive immunity [30]. In
64 contrast, the methylation dynamics of NK cells—particularly in the context of
65 cytokine-driven activation or NCR receptor engagement—remain much less
66 comprehensively characterized. Existing insights largely derive from studies of
67 cytomegalovirus (CMV) infection, wherein NK cells can undergo clonal-like
68 expansions resembling adaptive immune responses. In CMV-infected mice and
69 humans, NK cells expressing the NKG2C receptor have been observed to proliferate
70 extensively, forming memory-like subsets with unique phenotypic and
71 transcriptional characteristics. Intriguingly, many of these cells lack signaling
72 molecules typically associated with B-cell and myeloid pathways, such as the
73 tyrosine kinase SYK; this loss correlates with hypermethylation within a defined
74 promoter region of the SYK gene [15]. Additional methylation-associated
75 alterations have been documented in HCMV-associated NK cells, including
76 hypermethylation of signaling adaptors such as EAT-2 and FCER1G, as well as the
77 transcription factor ZBTB16/PLZF [23]. These modifications suggest that NK cells
78 possess a previously underappreciated capacity for epigenetic reprogramming in
79 response to persistent viral stimuli. Collectively, these findings prompted us to
80 undertake a detailed analysis aimed at identifying methylation loci that participate
81 specifically in NK-cell activation and may therefore serve as functional regulators
82 or biomarkers of NK responsiveness.
83 Despite increasing recognition of DNA methylation as a key regulator of immune
84 cell differentiation and lineage stability, the epigenetic mechanisms underlying NK
85 cell activation remain poorly defined. Most prior studies have focused on lineage-
86 specific methylation signatures or on specialized contexts such as cytomegalovirus-
87 associated adaptive NK-cell responses, leaving the broader landscape of activation-

88 associated methylation dynamics largely unexplored. The objective of the present
89 study is therefore to identify CpG loci whose methylation status changes during NK-
90 cell activation and to assess their potential functional relevance as regulators or
91 stable biomarkers of NK-cell responsiveness. By systematically characterizing
92 methylation alterations associated with NK-cell activation, this work aims to define
93 epigenetic signatures that distinguish activated from resting NK cells. The novelty
94 of this study lies in providing a focused analysis of activation-associated DNA
95 methylation patterns within the NK-cell compartment, thereby extending current
96 understanding of NK-cell biology from lineage-defining epigenetic states toward the
97 dynamic epigenetic regulation of innate immune effector functions. All the above
98 introduction was summarized in Fig. 1.

99 **Scientific Novelty**

100 Prior studies have reported gene-associated differentially methylated sites in
101 IL1RN, ECE1, CSF2, and DCHS1, indicating their involvement in epigenetic
102 regulation. However, the novelty of our study lies in the identification of previously
103 unreported CpG loci specifically associated with NK cell activation. By examining
104 methylation patterns in this biological context and applying an integrated analytical
105 framework, our work provides new insight into how epigenetic variation in these
106 genes may contribute to the regulation of NK cell functional responses, while also
107 refining and extending previous observations.

108 **2 Materials and Methods**

109 Epigenomic datasets generated from both naïve and activated NK cell
110 populations [28]—initially compiled to uncover genomic regions potentially
111 involved in NK cell-specific activation pathways—served as the foundation for our
112 reanalysis. These datasets were subjected to a systematic and multi-step
113 computational workflow designed to detect subtle yet functionally meaningful
114 epigenetic alterations. First, methylation delta values were calculated for every CpG

115 site to quantify the direction and magnitude of methylation changes occurring upon
116 NK cell activation. This calculation enabled the identification of CpG loci exhibiting
117 statistically significant shifts, thus highlighting epigenetic regions that respond
118 dynamically to activation stimuli. Following this quantitative assessment, each
119 differentially methylated CpG site was examined within the broader context of
120 genomic organization. Sites were mapped to their corresponding genes, promoters,
121 enhancers, and regulatory regions, allowing us to determine whether methylation
122 changes were positioned near elements known to influence transcriptional activity.
123 To deepen our interpretation, these genomic annotations were further integrated with
124 an extensive review of functional literature related to NK cell biology, cytokine
125 signaling, receptor-mediated activation, and inflammation-related gene networks.
126 This cross-referencing step enabled us to evaluate not only the molecular identity of
127 each locus but also its potential involvement in established NK cell pathways,
128 effector functions, or regulatory checkpoints. Through this comprehensive and
129 integrative analytic framework, we were able to distinguish activation-associated
130 loci from background variation and to infer mechanistic relevance for the most
131 prominent differentially methylated regions. In doing so, our approach provided
132 both a high-resolution view of activation-induced epigenetic remodeling and a
133 biologically informed interpretation of how these changes may contribute to the fine-
134 tuning of NK cell responses during immune activation.

135 **3 Results and Discussion**

136 Our analysis revealed four markedly significant differentially methylated sites
137 (DMSs) that were uniquely altered in activated NK cells and did not exhibit
138 comparable methylation shifts in any of the other immune cell types examined.
139 These activation-specific DMSs corresponded to cg11783497 (IL1RN),
140 cg07996532 (ECE1), cg17566874 (CSF2), and cg05550420 (DCHS1). The
141 exclusivity of these methylation changes to NK cells suggests the presence of a
142 specialized, cell-intrinsic epigenetic program that becomes engaged upon activation

143 and that functions to calibrate several essential aspects of NK cell biology, including
144 inflammatory output, signalling thresholds, and the capacity for tissue localization
145 and migration. By selectively modulating the methylation state of genes that regulate
146 cytokine activity, enzymatic processing, and cellular adhesion, NK cells appear to
147 employ an adaptive framework that safeguards the balance between potent effector
148 function and the prevention of excessive or dysregulated immune responses.
149 Previous epigenetic studies of NK cells have primarily focused on lineage identity
150 or virus-induced adaptive-like NK subsets. For example, CMV-associated NK cells
151 exhibit stable methylation changes in genes involved in signalling pathways,
152 including SYK, FCER1G, and ZBTB16/PLZF, reflecting long-term functional
153 reprogramming during chronic viral infection [15, 23]. Similarly, genome-wide
154 methylation analyses of immune cell populations have shown that lineage
155 specification in NK cells is accompanied by stable hypomethylation at loci such as
156 NCR1/NKp46 and other NK-associated regulatory elements [1]. However,
157 comparatively little is known about the CpG sites that undergo methylation changes
158 during NK-cell activation itself, particularly outside the context of viral infection or
159 adaptive-like NK differentiation. Our findings therefore extend previous work by
160 identifying specific activation-associated methylation loci that appear to be
161 selectively altered in NK cells and that are not shared by other immune cell
162 populations examined in this study. The first of these loci, IL1RN, encodes the
163 interleukin-1 receptor antagonist, a key anti-inflammatory molecule that
164 competitively inhibits signalling by IL-1 α and IL-1 β [25]. Although IL1RN is most
165 prominently produced by classical innate immune cells such as monocytes,
166 macrophages, and dendritic cells, its expression has also been documented in several
167 lymphoid subsets, including NK cells, where it may serve as part of an intrinsic
168 mechanism to fine-tune responsiveness [24]. Because IL-1 signalling is known to
169 augment NK cell cytotoxicity and cytokine secretion [26], the differential
170 methylation observed at the IL1RN locus in activated NK cells may represent an
171 epigenetically encoded negative-feedback mechanism. Earlier studies of IL-1

172 signalling in NK cells have largely emphasized cytokine-driven transcriptional
173 activation and receptor-mediated signalling pathways [26], whereas the present
174 results suggest that DNA methylation may represent an additional regulatory layer
175 that modulates IL-1-dependent NK-cell responses. The second locus, ECE1,
176 encodes endothelin-converting enzyme-1, a metalloprotease responsible for
177 catalyzing the conversion of the precursor form of endothelin-1 (ET-1) into its fully
178 active peptide [22]. Active ET-1 is recognized as a potent proinflammatory mediator
179 capable of stimulating canonical inflammatory pathways such as NF- κ B and
180 inducing the production of cytokines including TNF- α , IL-1, and IL-6 [14]. Most
181 previous research on ECE1 has focused on its role in vascular physiology and
182 inflammatory signalling in endothelial or cardiovascular systems [14,22], and its
183 potential involvement in immune-cell epigenetic regulation has received little
184 attention. The observation that ECE1 undergoes differential methylation specifically
185 in activated NK cells therefore suggests that NK cells may employ epigenetic
186 modulation of endothelin-related pathways to limit excessive inflammatory
187 signalling during immune activation. The third significant locus, CSF2, encodes
188 granulocyte–macrophage colony-stimulating factor (GM-CSF), a multifunctional
189 cytokine involved in the development, activation, and survival of granulocytic
190 lineages, including neutrophils, eosinophils, and basophils [8]. CSF2 expression can
191 be induced in several immune cell types—among them NK cells and Th17 cells—
192 in response to inflammatory stimuli or cytokine stimulation [18]. Prior studies have
193 demonstrated that NK-cell-derived GM-CSF production typically occurs under
194 strong activating conditions, particularly in response to cytokines such as IL-18 [8].
195 However, these investigations have largely addressed transcriptional regulation of
196 CSF2 expression rather than epigenetic control. The activation-associated
197 methylation changes identified at this locus therefore suggest that NK cells may use
198 DNA methylation to establish regulatory thresholds for GM-CSF secretion, thereby
199 preventing excessive recruitment and activation of downstream myeloid effector
200 populations. The final locus identified, DCHS1, encodes a cadherin-family adhesion

201 protein containing numerous cadherin repeat domains and a distinctive cytoplasmic
202 tail that enables participation in diverse cell–cell and tissue-structural interactions.
203 DCHS1 has been implicated in mammalian tissue morphogenesis, maintenance of
204 progenitor cell populations, and regulation of cellular behaviours associated with
205 planar cell polarity and coordinated migration [20]. Most existing studies have
206 examined DCHS1 primarily in developmental or tissue-organization contexts rather
207 than in immune-cell function [20]. The differential methylation of the DCHS1 locus
208 observed specifically in activated NK cells therefore suggests a potentially novel
209 link between adhesion-related pathways and NK-cell activation. Because NK cells
210 depend on controlled migration and tissue infiltration to identify infected or
211 transformed targets, methylation-dependent modulation of DCHS1 may influence
212 their ability to detach, migrate, and reposition within inflamed tissues. Taken
213 together, these findings complement previous research demonstrating that NK cells
214 undergo epigenetic remodeling during differentiation and during virus-induced
215 adaptive-like responses [15,23]. However, in contrast to earlier studies that
216 emphasized lineage-defining methylation patterns or infection-associated epigenetic
217 reprogramming, the present analysis identifies activation-specific CpG loci uniquely
218 altered in NK cells and associated with pathways regulating inflammatory
219 signalling, cytokine production, and cellular migration. The principal novelty of this
220 work therefore lies in the identification of a previously unrecognized set of NK-cell-
221 specific DMSs linked to IL1RN, ECE1, CSF2, and DCHS1, which may represent
222 candidate epigenetic regulators or biomarkers of NK-cell activation. These findings
223 broaden the current understanding of NK-cell epigenetic regulation by
224 demonstrating that NK-cell activation is accompanied not only by transient
225 transcriptional changes but also by targeted DNA-methylation modifications that
226 may help stabilize functional NK-cell responses and maintain immune homeostasis.
227 All the above discussed data are summarized in Table 1.

228 **4 Conclusions**

229 Building upon the results of our analysis, it is reasonable to propose that the
230 differentially methylated genes identified in activated NK cells—namely IL1RN,
231 ECE1, CSF2, and DCHS1—may undergo either complete or partial transcriptional
232 repression as part of a regulatory mechanism that limits excessive immune
233 activation. DNA methylation is widely recognized as a mechanism capable of
234 stabilizing transcriptional silencing and shaping immune-cell functional states. In
235 the context of NK cells, most previously described methylation changes have been
236 linked to lineage identity or to adaptive-like NK-cell responses associated with
237 chronic viral infection. For instance, epigenetic remodelling in CMV-associated NK
238 cells includes stable hypermethylation of genes such as SYK, FCER1G, and
239 ZBTB16, reflecting long-term functional reprogramming of NK-cell signalling
240 pathways. Similarly, other studies have shown that NK-cell lineage commitment is
241 accompanied by hypomethylation at loci such as NCR1/NKp46, which functions as
242 a stable epigenetic marker of NK-cell identity. In contrast to these earlier findings,
243 which primarily describe methylation signatures associated with differentiation or
244 infection-driven adaptive NK-cell subsets, the present study focuses specifically on
245 epigenetic changes occurring during NK-cell activation itself. Our results indicate
246 that activation of NK cells is accompanied by selective methylation changes at genes
247 involved in inflammatory regulation (IL1RN), enzymatic control of inflammatory
248 mediators (ECE1), cytokine signalling (CSF2), and adhesion-related cellular
249 behaviour (DCHS1). While previous studies have documented transcriptional
250 activation of cytokine pathways and signalling cascades during NK-cell stimulation,
251 the potential role of DNA methylation in modulating these pathways during
252 activation has remained largely unexplored. The identification of methylation
253 changes in IL1RN suggests the existence of an epigenetic mechanism capable of
254 modulating IL-1–dependent inflammatory amplification, which has previously been
255 studied mainly at the level of cytokine signalling and receptor interactions.
256 Similarly, the detection of differential methylation at the ECE1 locus extends earlier
257 research that has primarily associated endothelin-converting enzyme-1 with vascular

258 inflammation and endothelial signalling rather than immune-cell regulation. The
259 activation-associated methylation of CSF2 indicates that NK cells may employ
260 epigenetic constraints to regulate GM-CSF production, complementing previous
261 work demonstrating cytokine-induced transcriptional expression of this gene in NK
262 and T helper cells. Finally, the involvement of DCHS1, a cadherin-family adhesion
263 molecule mainly studied in tissue morphogenesis and planar cell polarity, points to
264 a potential epigenetic mechanism influencing NK-cell migration and tissue
265 positioning during immune responses—an aspect that has not been widely addressed
266 in earlier NK-cell epigenetic studies. Taken together, these observations distinguish
267 the present findings from previous work by demonstrating that NK-cell activation is
268 associated with targeted DNA methylation changes affecting genes that regulate
269 inflammatory signalling, cytokine production, and cellular motility. Whereas earlier
270 studies emphasized long-term epigenetic remodeling during viral infection or
271 lineage differentiation, the current results suggest that activation itself can be
272 accompanied by specific and potentially functional methylation modifications. The
273 novelty and significance of this study therefore lie in the identification of a
274 previously unrecognized set of activation-associated epigenetic markers—IL1RN,
275 ECE1, CSF2, and DCHS1—that may contribute to controlling NK-cell
276 responsiveness. These loci may represent candidate regulatory nodes through which
277 NK cells fine-tune their activation threshold, thereby preserving immune
278 homeostasis while maintaining the capacity for rapid and effective cytotoxic
279 responses.

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281 version of the manuscript.

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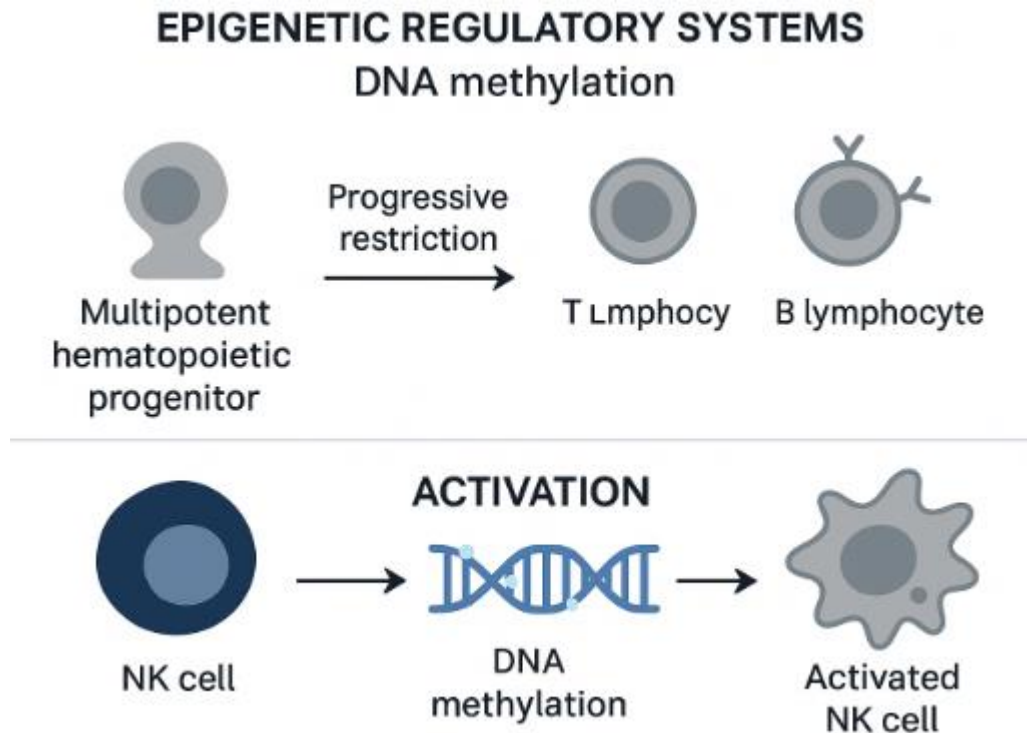
ТАБЛИЦЫ

Table 1. Activation-Specific Differentially Methylated Sites (DMSs) in NK Cells and Their Functional Roles

CpG ID / Gene	Gene Function	Biological Role in Immunity	Interpretation of NK-Cell-Specific Differential Methylation
cg11783497 (IL1RN)	Encodes IL-1 receptor antagonist (IL-1Ra), an inhibitor of IL-1 α/β signaling	Limits IL-1-driven inflammatory amplification; expressed mainly in monocytes/macrophages but also detectable in NK cells	Likely represents an epigenetically encoded negative-feedback mechanism to prevent runaway IL-1-mediated NK-cell activation, maintaining proportional cytotoxic and cytokine responses
cg07996532 (ECE1)	Encodes endothelin-converting enzyme-1 (ECE-1), which activates endothelin-1 (ET-1)	ET-1 promotes proinflammatory signaling (NF- κ B activation; induction of TNF- α , IL-1, IL-6)	Methylation-linked repression may limit ET-1 activation, preventing inadvertent initiation of broad inflammatory

			cascades and preserving tissue homeostasis during NK-cell activation
cg17566874 (CSF2)	Encodes GM-CSF, a cytokine that promotes development and activation of myeloid cells	GM-CSF production by NK cells occurs mainly under strong inflammatory cues (e.g., IL-18); shapes myeloid recruitment and activation	Activation-specific methylation likely prevents premature GM-CSF secretion, avoiding excessive myeloid cell recruitment and maintaining control over inflammatory microenvironment restructuring
cg05550420 (DCHS1)	Encodes a cadherin-family adhesion molecule involved in cell–cell interaction, tissue morphogenesis, and migration	Regulates cellular adhesion, motility, and structural organization in tissues	Differential methylation may enable adaptive remodeling of adhesion and migration pathways, enhancing NK-cell trafficking, tissue repositioning, and efficient immune surveillance during activation

РИСУНКИ

Figure 1. Epigenetic regulatory systems.

ТИТУЛЬНЫЙ ЛИСТ_МЕТАДААННЫЕ

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Блок 3. Метаданные статьи

DIFFERENTIAL DNA METHYLATION OF NK CELLS PREVENTS THEIR
EXCESSIVE ACTIVATION

ДИФФЕРЕНЦИАЛЬНАЯ МЕТИЛИРОВАНИЕ ДНК ПРЕДОТВРАЩАЕТ
ЧРЕЗМЕРНУЮ АКТИВАЦИЮ НК-КЛЕТОК

Сокращенное название статьи для верхнего колонтитула:

NK CELLS DIFFERENTIAL DNA METHYLATION

ДИФФЕРЕНЦИАЛЬНАЯ МЕТИЛИРОВАНИЕ ДНК В НК-КЛЕТКАХ

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Epigenetics, Genes.

Ключевые слова: НК-клетки, Иммунная система, Метилирование ДНК,
Иммунология, Эпигенетика, Гены.

Обзоры.

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СПИСОК ЛИТЕРАТУРЫ

Reference sequence number	Authors, title of a publication and source where it was published, publisher's imprint	Full name, title of a publication and source in English	Reference's URL
1	Calvanese V, Fernandez AF, Urduingio RG, Suarez-Alvarez B, Mangas C, Perez-Garcia V, Bueno C, Montes R, Ramos-Mejia V, Martinez-Cambolor P, et al. A promoter DNA demethylation landscape of human hematopoietic differentiation. Nucleic Acids Res 2012; 40:116-31; PMID:21911366;	—	http://dx.doi.org/10.1093/nar/gkr685
2	Bird A. DNA methylation patterns and epigenetic memory. Genes Dev 2002; 16:6-21; PMID:11782440;	—	http://dx.doi.org/10.1101/gad.947102
3	Yu Y, Mo Y, Ebenezer D, Bhattacharyya S, Liu H, Sundaravel S, Giricz O, Wontakal S, Cartier J, Caces B, et al.	—	http://dx.doi.org/10.1074/jbc.M112.423756

	High resolution methylome analysis reveals widespread functional hypomethylation during adult human erythropoiesis. J Biol Chem 2013; 288:8805-14; PMID:23306203;		
4	Karpurapu M, Ranjan R, Deng J, Chung S, Lee YG, Xiao L, Nirujogi TS, Jacobson JR, Park GY, Christman JW. Kruppel like factor 4 promoter undergoes active demethylation during monocyte/macrophage differentiation. PloS one 2014; 9: e93362; PMID:24695324;	—	http://dx.Doi.org/10.1371/journal.pone.0093362
5	Kitagawa Y, Ohkura N, Sakaguchi S. Molecular determinants of regulatory T cell development: the essential roles of epigenetic changes. Front Immunol 2013; 4:106; PMID:23675373;	—	http://dx.doi.org/10.3389/fimmu.2013.00106
6	Komori HK, Hart T, LaMere SA, Chew PV, Salomon DR. Defining CD4 T cell memory by the epigenetic landscape of	—	http://dx.doi.org/10.4049/jimmunol.1401162

	CpG DNA methylation J Immunol 2015; 194:1565-79; PMID:25576597;		
7	Lee ST, Xiao Y, Muench MO, Xiao J, Fomin ME, Wiencke JK, Zheng S, Dou X, de Smith A, Chokkalingam A, et al. A global DNA methylation and gene expression analysis of early human B-cell development reveals a demethylation signature and transcription factor network. Nucleic Acids Res 2012; 40:11339-51; PMID:23074194;	—	http://dx.doi.org/10.1093/nar/gks957
8	Wieczorek G, Asemissen A, Model F, Turbachova I, Floess S, Liebenberg V, Baron U, Stauch D, Kotsch K, Pratschke J, et al. Quantitative DNA methylation analysis of FOXP3 as a new method for counting regulatory T cells in peripheral blood and solid tissue. Cancer Res 2009; 69:599-608; PMID:19147574;	—	http://dx.doi.org/10.1158/0008-5472.CAN-08-2361

9	Accomando WP, Wiencke JK, Houseman EA, Butler RA, Zheng S, Nelson HH, Kelsey KT. Decreased NK cells in patients with head and neck cancer determined in archival DNA. Clin Cancer Res 2012; 18:6147-54; PMID:23014525;	—	http://dx.doi.org/10.1158/1078-0432.CCR-12-1008
10	Best JA, Blair DA, Knell J, Yang E, Mayya V, Doedens A, Dustin ML, Goldrath AW, Immunological Genome Project C. Transcriptional insights into the CD8(C) T cell response to infection and memory T cell formation. Nat Immunol 2013; 14:404-12; PMID:23396170;	—	http://dx.doi.org/10.1038/ni.2536
11	Bezman NA, Kim CC, Sun JC, Min-Oo G, Hendricks DW, Kamimura Y, Best JA, Goldrath AW, Lanier LL, Immunological Genome Project C. Molecular definition of the identity and activation of natural killer cells. Nat Immunol 2012; 13:1000-9; PMID:22902830;	—	http://dx.doi.org/10.1038/ni.2395

12	Dybkaer K, Iqbal J, Zhou G, Geng H, Xiao L, Schmitz A, d'Amore F, Chan WC. Genome wide transcriptional analysis of resting and IL2 activated human natural killer cells: gene expression signatures indicative of novel molecular signaling pathways. BMC Genomics 2007; 8:230; PMID:17623099;	—	http://dx.doi.org/10.1186/1471-2164-8-230
13	Hu G, Chen J. A genome-wide regulatory network identifies key transcription factors for memory CD8(C) T-cell development. Nat Commun 2013; 4:2830; PMID:24335726;	—	http://doi:10.1038/ncomms3830
14	Ma D, Cao W, Kapur A, Felder M, Scarlett CO, Patankar MS, Li L. Differential expression of proteins in naive and IL-2 stimulated primary human NK cells identified by global proteomic analysis. J Proteomics 2013; 91:151-63; PMID:23806757;	—	http://dx.doi.org/10.1016/j.jpro.2013.06.024
15	Melvin AJ, McGurn ME, Bort SJ, Gibson C, Lewis DB. Hypomethylation of the interferon-gamma gene correlates	—	http://dx.doi.org/10.1002/eji.1830250218

	with its expression by primary T-lineage cells. Eur J Immunol 1995; 25:426-30; PMID:7875204;		
16	Bruniquel D, Schwartz RH. Selective, stable demethylation of the interleukin-2 gene enhances transcription by an active process. Nat Immunol 2003; 4:235-40; PMID:12548284;	—	http://dx.doi.org/10.1038/ni887
17	Harland KL, Day EB, Apte SH, Russ BE, Doherty PC, Turner SJ, Kelso A. Epigenetic plasticity of Cd8a locus during CD8(C) T-cell development and effector differentiation and reprogramming. Nat Commun 2014; 5:3547; PMID:24675400;	—	http://dx.doi.org/10.1038/ncomms4547
18	Li Y, Chen G, Ma L, Ohms SJ, Sun C, Shannon MF, Fan JY. Plasticity of DNA methylation in mouse T cell activation and differentiation. BMC Mol Biol 2012; 13:16; PMID:22642378;	—	http://dx.doi.org/10.1186/1471-2199-13-16

19	Zhang X, Ulm A, Somineni HK, Oh S, Weirauch MT, Zhang HX, Chen X, Lehn MA, Janssen EM, Ji H. DNA methylation dynamics during ex vivo differentiation and maturation of human dendritic cells. <i>Epigenetics Chromatin</i> 2014; 7:21; PMID:25161698;	—	http://dx.doi.org/10.1186/1756-8935-7-21
20	Lee J, Zhang T, Hwang I, Kim A, Nitschke L, Kim M, Scott JM, Kamimura Y, Lanier LL, Kim S. Epigenetic Modification and Antibody-Dependent Expansion of Memory-like NK Cells in Human Cytomegalovirus-Infected Individuals. <i>Immunity</i> 2015; 42:431-42; PMID:25786175;	—	http://dx.doi.org/10.1016/j.immuni.2015.02.013
21	Schlums H, Cichocki F, Tesi B, Theorell J, Beziat V, Holmes TD, Han H, Chiang SC, Foley B, Mattsson K, et al. Cytomegalovirus infection drives adaptive epigenetic diversification of NK cells with altered signaling and effector function. <i>Immunity</i> 2015; 42:443-56;		

22	<p>Wiencke JK, Butler R, Hsuang G, Eliot M, Kim S, Sepulveda MA, Siegel D, Houseman EA, Kelsey KT. The DNA methylation profile of activated human natural killer cells. <i>Epigenetics</i>. 2016 May 3;11(5):363-80. doi: 10.1080/15592294.2016.1163454. Epub 2016 Mar 11. PMID: 26967308; PMCID: PMC4889279.</p>		
23	<p>Shiiba M, Saito K, Yamagami H, Nakashima D, Higo M, Kasamatsu A, Sakamoto Y, Ogawara K, Uzawa K, Takiguchi Y, Tanzawa H. Interleukin-1 receptor antagonist (IL1RN) is associated with suppression of early carcinogenic events in human oral malignancies. <i>Int J Oncol</i>. 2015 May;46(5):1978-84. doi: 10.3892/ijo.2015.2917. Epub 2015 Mar 4. PMID: 25738940.</p>		

24	<p>Shanley TP, Hallstrom C, Wong HR, Chapter 96 - Sepsis, Editor(s): FuhrmanBP , Zimmerman JJ, Pediatric Critical Care (Third Edition), Mosby, 2006, Pages 1474-1493, doi: 10.1016/B978-032301808-1.50099-7.</p>		
25	<p>Van Den Eeckhout B, Tavernier J, Gerlo S. Interleukin-1 as Innate Mediator of T Cell Immunity. Front Immunol. 2021 Jan 27;11:621931. doi: 10.3389/fimmu.2020.621931. PMID: 33584721; PMCID: PMC7873566.</p>		
26	<p>Kowalczyk A, Kleniewska P, Kolodziejczyk M, Skibska B, Goraca A. The role of endothelin-1 and endothelin receptor antagonists in inflammatory response and sepsis. Arch Immunol Ther Exp (Warsz). 2015 Feb;63(1):41-52. Epub 2014 Oct 7. PMID: 25288367; PMCID: PMC4289534.</p>		<p>http://dx.doi.org/10.1007/s00005-014-0310-1.</p>

27	<p>Padilla BE, Cottrell GS, Roosterman D, Pikios S, Muller L, Steinhoff M, Bunnett NW. Endothelin-converting enzyme-1 regulates endosomal sorting of calcitonin receptor-like receptor and beta-arrestins. <i>J Cell Biol.</i> 2007 Dec 3;179(5):981-97. Epub 2007 Nov 26. PMID: 18039931; PMCID: PMC2099187.</p>		<p>http://dx.doi.org/10.1083/jcb.200704053</p>
28	<p>Louis C, Souza-Fonseca-Guimaraes F, Yang Y, D'Silva D, Kratina T, Dagley L, Hediye-Zadeh S, Rautela J, Masters SL, Davis MJ, Babon JJ, Ciric B, Vivier E, Alexander WS, Huntington ND, Wicks IP. NK cell-derived GM-CSF potentiates inflammatory arthritis and is negatively regulated by CIS. <i>J Exp Med.</i> 2020 May 4;217(5):e20191421. Erratum in: <i>J Exp Med.</i> 2020 May 4;217(5):e2019142103192020c.</p>		<p>http://dx.doi.org/10.1084/jem.20191421</p>

	doi: 10.1084/jem.2019142103192020c. PMID: 32097462; PMCID: PMC7201918.		
29	Francisco-Cruz A, Aguilar-Santelises M, Ramos-Espinosa O, Mata-Espinosa D, Marquina-Castillo B, Barrios-Payan J, Hernandez-Pando R. Granulocyte-macrophage colony-stimulating factor: not just another haematopoietic growth factor. Med Oncol. 2014 Jan;31(1):774. Epub 2013 Nov 22. PMID: 24264600.		http://dx.doi.org/10.1007/s12032-013-0774-6 .
30	Meijuan C, Fang M, Qian W. Dachsous cadherin related 1 (DCHS1) is a novel biomarker for immune infiltration and epithelial-mesenchymal transition in endometrial cancer via pan-cancer analysis. J Ovarian Res. 2024 Aug 9;17(1):162. PMID: 39123216; PMCID: PMC11312386.		http://dx.doi.org/10.1186/s13048-024-01478-1

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