MicroRNAs as tools to predict glucocorticoid response in inflammatory bowel diseases

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Abstract

In spite of the introduction in therapy of highly effective biological agents, glucocorticoids (GCs) are still employed to induce remission in moderate to severe inflammatory bowel diseases (IBD), but considerable inter-individual differences in their efficacy and side effects have been reported. The effectiveness of these drugs is indeed very variable and side effects, particularly severe in pediatric patients, are common and often unpredictable: the understanding of the complex gene regulation mediated by GCs could shed light on the causes of this variability. In this context, microRNAs (miRNAs) represent a new and promising field of research. miRNAs are small non-coding RNA molecules that suppress gene expression at post-transcriptional level, and are fine-tuning regulators of diverse biological processes, including the development and function of the immune system, apoptosis, metabolism and inflammation. Emerging data have implicated the deregulated expression of certain miRNA networks in the pathogenesis of autoimmune and inflammatory diseases, such as IBD. There is a great interest in the identification of the role of miRNAs in the modulation of pharmacological response; however, the association between miRNA and GC response in patients with IBD has not yet been evaluated in a prospective clinical study. The identification of miRNAs differently expressed as a consequence of GC treatment in comparison to diagnosis, represents an important innovative approach that could be translated into clinical practice. In this review we highlight the altered regulation of proteins involved in GC molecular mechanism by miRNAs, and their potential role as molecular markers useful for predicting in advance GC response.

Key words: Glucocorticoids; Inflammatory bowel diseases; MicroRNA; Molecular markers; Pharmacogenomics

Core tip: Studies on microRNAs (miRNAs) and pharmacogenomics represent a promising investigation topic that could increase the understanding of the pharmacology of steroids in inflammatory bowel diseases (IBDs) and possibly in other diseases. A number of studies have shown that glucocorticoids (GCs) can modify the expression profiles of different miRNAs, however, the obtained results have been highly variable, and to date it is not possible to recognize a specific miRNA pattern regulated by GCs. Moreover, existing studies employed techniques based on the use of reverse transcription quantitative polymerase chain reaction and microarrays, through the analysis and quantification of already known miRNAs. Using next generation sequencing technologies, it could be possible to detect novel, still unrecognised miRNAs, and identify new miRNA isofoms (iso-miRs) as well. This innovative approach could be a valuable tool for a better understanding of the role...
of miRNAs to predict steroid response in IBDs. In the future, the increased availability and the reduced costs of RNA profiling should enable the clinicians to stratify patients on specific miRNA biomarkers before starting GC treatment.

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INTRODUCTION

To date, a curative pharmacological therapy for inflammatory bowel diseases (IBD) does not exist and the therapeutic approach is mainly aimed at controlling inflammation, with drugs capable of inducing and maintaining remission. Despite the introduction in therapy of highly effective biological agents, in IBD patients moderate to severe disease glucocorticoids (GCs) are effective in inducing remission and are still considered the standard for treatment[1]. In spite of the large clinical use, the benefits of these agents are often narrowed by high inter-individual variability. Given the high incidence of suboptimal response, associated with a significant number of side effects, the identification of subjects that are most likely to respond poorly to these agents is extremely important. However, the mechanisms of this variability are scarcely understood and there is presently no means to predict the response in advance[2-5]; in this context, microRNAs (miRNAs) represent a new and promising field of research.

miRNAs are small (18-24 nucleotides) non-coding RNAs, which bind the 3'UTRs and the coding exons of their target genes and inhibit gene expression[6] either by messenger RNA (mRNA) cleavage (most common in plants) or by translational repression (most common in metazoan)[7]. According to the miRNA database miRBase, 1872 precursors and 2578 human mature miRNA sequences have been published (http://www.mirbase.org)[8], and we are only on the verge of understanding their physiological impact on gene regulation. A single miRNA can regulate a multitude of mRNAs (approximately 200), and each mRNA can be regulated by multiple miRNAs[9,10]; overall, it is predicted that protein production for at least 20% of all human genes is regulated by miRNAs[11,12].

By affecting gene regulation, miRNAs are likely to be implicated in the control of diverse biological processes, such as cellular proliferation and apoptosis[13,14], stem cell differentiation[15,18-20], and organ development and morphogenesis[21,22]; in addition a strong association between miRNA expression dysregulation and induction of cancer has been shown[23-26]. Moreover, miRNAs have important regulatory roles in the innate and adaptive immune system[27-29], and characteristic miRNA expression profiles have been demonstrated even in IBD[30-33].

There is a lot of interest in identifying the role of miRNAs in the modulation of drug response[34], but studies about this topic are still very limited, and the possible correlation between miRNAs expression and variability on GC response in IBD patients has not yet been examined. A better knowledge of miRNAs role could lead to their use as biomarkers for IBD, and consequently, to the development of new strategies for therapy personalization in these diseases.

This review tries to highlight the altered regulation of proteins involved in GC molecular mechanism by miRNAs in different diseases and in vitro models, and their potential role as molecular markers useful for predicting in advance GC response.

GLUCOCORTICOIDS IN INFLAMMATORY BOWEL DISEASES

GCs are effective inhibitors of cytokine secretion and T-cell activation, and are consequently largely employed in different inflammatory conditions, including IBD. Despite the introduction of novel therapies, these agents are still currently used for induction of remission in moderate to severe IBDs, however, a wide variability in response to these agents is evident and, in these diseases, GC resistance or dependence is particularly frequent. Among the adult IBD population, a prospective analysis has described the 1-year outcome in patients with Crohn’s disease (CD) treated with a first oral prednisone course (40-60 mg/d) and tapering to a maintenance dose of 10-15 mg/d[35]. Prolonged steroid response was obtained in 44% of patients, 36% of subjects were steroid dependent while 20% of subjects did not respond and were steroid resistant; a high frequency of surgery was reported within 1 mo after steroid treatment. Similar results have been obtained in a retrospective American study: immediate outcomes for CD and ulcerative colitis (UC), respectively, were complete remission in 58% and 54% of cases, partial remission in 26% and 30%, resistance in 16% of patients[36]. In paediatric IBD patients, clinical reports have shown that up to 90% of subjects has a rapid improvement of symptoms when prednisone treatment is given; however, after 1 year, only 55% of patients were still in remission and were considered steroid responsive. In around 38% of patients, steroid therapy could not be discontinued as patients experienced an increase of disease activity when the dose was reduced (steroid dependent)[37].

Demographic and/or clinical markers[38,39,40] have been evaluated and related with this variability in GC response, but results have not been consistently replicated. Genetic and epigenetic markers are likely to complement clinical and demographic predictors: phenotypes resulting from genetic changes and regulation can markedly influence drug pharmacokinetics or alter drug efficacy and/or toxicity profiles. The identification of genetic biomarkers that can be useful for classifying the disease and help to improve therapy is paramount.
MOLECULAR MECHANISM OF GC ACTION

The effects of GCs are mediated by the glucocorticoid receptor (GR-α), a member of the nuclear receptor superfamily of ligand-dependent transcription factors[40,41]. The human GR gene is encoded on chromosome 5q31.3 and consists of nine coding exons[42]. Alternative splicing of exon 9 generates two receptor isoforms, GR-α and GR-β[43-46]. GR-β is not able to bind GCs, resides constitutively in the nucleus of cells, has a longer half-life than GR-α, and does not transactivate GC-inducible reporter genes[47]. It has been suggested[48,49] that cell specific expression and function of GR isoforms may explain the tissue and individual selective actions of GCs.

The function of GR is conditioned by chaperone and co-chaperone proteins that form a molecular heterocomplex with the GR itself[50,51], required for proper ligand binding, receptor activation and transcription: abnormalities in proteins that make up the heterocomplex may contribute to altered GC responsiveness[52,53]. Several studies have demonstrated differences in the heterocomplex gene expression profiles in steroid resistant in comparison with responder patients, but it is not clear if this different expression is the cause of the variability in response or the consequence of GC treatment[54-56]. After GC binding and dissociation from heterocomplex proteins, the GR translocates into the nucleus; translocation is mediated by specific nuclear transport factors that belong to the importin β family of nuclear transporters, and in particular by importin 13[57]. The activated receptor then binds as homodimer two palindromic DNA-binding sites, the so-called glucocorticoid responsive elements (GREs), localized in the promoter region of target genes[60-63]. As a consequence of DNA binding, GCs can induce trans-activation and trans-repression processes: binding to positive GREs leads to activation of the transcription of anti-inflammantory [e.g., interleukin 10 (IL-10), Annexin 1] as well as of regulator proteins involved in metabolic processes [e.g., enzymes of gluconeogenesis][64-66]. The second mechanism of GC action is trans-repression[67], which leads to a reduced expression of immune-regulatory and proinflammatory proteins such as cytokines [IL-1, IL-2, IL-6, tumor necrosis factor-α (TNF-α)] and prostaglandins[68], and is believed to be responsible for the majority of beneficial anti-inflammatory effects.

Steroid hormones can regulate gene expression posttranscriptionally, by destabilizing miRNAs[69]. In addition, these hormones can induce rapid non genomic effects within the cytoplasm; for example, they induce the release of Src kinase from the GR heterocomplex, resulting in lipocortin activation and inhibition of arachidonic acid release[70,71], and alter cytoplasmic ion content[72,73].

miRNAs AND GC RESPONSE

miRNA regulation by GCs

It has been demonstrated that activation of GR by GCs might induce or repress specific miRNAs in various target genes. The majority of studies have evaluated the effect of GCs on miRNA expression levels in tumor leukemic cells, during GC induced apoptosis[74].

Rainer et al[75] have correlated miRNA levels with expression data of their host genes in cell lines and clinical samples of children with acute lymphoblastic leukemia (ALL) undergoing systemic GC monotherapy. At least 5 miRNAs were significantly regulated by GC therapy. Importantly, the miR-15/16 cluster, which induces cell cycle arrest, was up-regulated by GCs in a subset of ALL patients and cell lines, consistent with the known apoptotic effect of GCs in immature lymphoblasts. Indeed, overexpression of miR-15b/16 increased GC sensitivity in leukemia cell lines whereas silencing miR-15b/16 with inhibitors decreased GC sensitivity in vitro.

Another study in a T-cell lymphoma cell line has shown that GC treatment repressed the expression of the miRNA cluster miR-17-92, which results in elevated protein expression of Bim, a proapoptotic member of the B-cell lymphoma-2 family (Bcl-2). Overexpression of miRNA cluster miR-17-92 decreased Bim induction, and attenuated GC mediated apoptosis, while cluster knockdown increased Bim induction and GC mediated apoptosis[76]. These findings suggest a novel mechanism that could contribute to the induction of lymphocyte apoptosis by GCs.

Harada et al[77] demonstrated that in the leukemic cell line RS4; 11 dexamethasone down-regulated miRNA levels; miR17HG was rapidly down-regulated, and chromatin immunoprecipitation demonstrated that the promoter is a target of GC transcriptional repression; in particular, the miR-17-92 cluster was identified as a prime target for dexamethasone induced repression. In the sensitive leukemia cell line SUP-B15, but not in the resistant line REH, dexamethasone reduced the expression of the miR-17 family and concomitantly increased its target protein Bim. Up-regulation or inhibition of miR-17 resulted in a decrease and increase, respectively in Bim protein levels and in dexamethasone induced cytotoxicity. Down-regulation of miR-17 levels was observed in ex vivo patients’ leukemia cells that underwent dexamethasone induced apoptosis[78].

Another recent study[79], by genome wide miRNA microarray on diagnostic bone marrow samples of ALL pediatric patients treated with GCs, identified a reduced expression of miR-355 as the most significant miRNA abnormality associated with poor outcome. Moreover, the authors demonstrated that exogenous expression of miR-355 in ALL cells increases sensitization to prednisolone-induced apoptosis. MAPK1 was identified as a target of miR-355, and the MEK/ERK inhibitor treatment increased GC induced cytotoxicity through the activation of Bim.

Smith et al[80] have demonstrated that miRNAs are repressed during GC induced apoptosis of primary rat thymocytes, and further demonstrated the repression
of the miRNA processing enzymes Dicer, Drosha and DGCR8/Pasha. Silencing of Dicer expression in two human leukemia lines significantly enhanced GC induced apoptosis, while overexpression of the GC-repressed miR-17-92 polycistron reduced apoptosis.

Among the few studies that have considered the effect of GCs on miRNA expression in non tumor cells, Ledderhose et al.\textsuperscript{[80]} in native and CD3/CD28 stimulated cells from healthy volunteers, demonstrated that miR-24 is expressed in human T cells, and expression is increased 1.7 fold upon stimulation. Hydrocortisone significantly enhanced by 3 fold the miRNA induction\textsuperscript{[80]}.

In human corneal fibroblast treated for 16 h with dexamethasone, genome microarray and miRNA analyses were used to evaluate gene and miRNA expression. In response to treatment with the steroid, 261 genes were up-regulated and 123 were down-regulated more than three-fold. Several miRNAs, including miR-16, miR-21 and miR-29c were up-regulated, whereas miR-100 was down-regulated by the steroid, suggesting a posttranscriptional control of gene expression through miRNAs\textsuperscript{[81]}.

Studies of the miRNAs profile on mucosal biopsies of patients with eosinophilic esophagitis, before and after successful treatment with GCs were conducted by Lu and collaborators\textsuperscript{[82]}; of the 377 miRNA sequences examined, 32 miRNAs were significantly up-regulated and 4 down-regulated in the biopsies obtained before treatment compared to samples obtained after GC therapy. miR-214 was the most up-regulated (150 fold) and miR-146b-5b, 146a, 145, 142-3p and 21 were up-regulated by at least 10 fold.

Williams et al.\textsuperscript{[83]}, using a highly sensitive reverse transcription-polymerase chain reaction, measured 277 miRNAs in airway biopsies obtained from normal subjects and mild asthmatic patients before and after one month twice daily treatment with inhaled budesonide. No significant difference in miRNA expression was evident in the airway biopsies of normal and asthmatic subjects, and, despite improved lung function, no change in miRNAs expression was evident after one month budesonide treatment. However, a specific miRNA expression profile was observed in different cell types (alveolar epithelial cells, airway smooth muscle cells, alveolar macrophages, lung fibroblasts).

Finally, in a recent study\textsuperscript{[84]}, activated human CD4\textsuperscript{+} T cells from healthy donors were exposed in vitro to 1 µmol/L of methylprednisolone and changes in mRNA and miRNA expression profiles were analyzed by microarrays; a number of steroid responsive genes and miRNAs were identified. Further studies with qPCR, flow cytometry and ELISA, demonstrated that methylprednisolone increased the expression of miR-98 and suppressed the levels of predicted targets, including the pro-inflammatory cytokine IL-13 and three TNF receptors FAS, FASL, and TNF receptor superfamily member 1B (TNFRSF1B); these data suggest that methylprednisolone acts through miR-98 to inhibit specific pro-inflammatory targets\textsuperscript{[84]}.

**GR as miRNA target**

The role of miRNAs in the regulation of the GR has been examined, indeed, computational studies showed that the 3’ UTR of the GR is predicted to contain numerous seed regions recognized by a variety of miRNAs\textsuperscript{[85]}.

Using a combination of in silico prediction of miRNA binding sites, miRNA overexpression studies and mutagenesis of the GR 3’UTR, Vreugdenhil and collaborators\textsuperscript{[86]} found that miR-18 and miR-124a bind GR mRNA and decrease GR activity in neuronal tissues. These miRNAs were tested for their ability to alter the translational activity of GR and reduce GR protein levels in cell cultures in vitro; miR-18 and miR-124a overexpression reduced GR protein levels and impaired the activation of the GR responsive gene glucocorticoid-induced leucine zipper (GILZ). In addition these authors have demonstrated by miRNA reporter assay that miR-124a is able to bind to the predicted seed region in the GR 3’ UTR.

Ledderhose et al\textsuperscript{[80]} have investigated the role of miR-124 in the regulation of GR expression; these authors have studied the influence of the GR isoforms (the active isoform α, and the dominant negative non-ligand-binding isoform β) on GC effects in human T-cells, and found that, in patients with critical illness-related corticosteroid insufficiency, miR-124 specifically down-regulated GR-α; a slight increase of miR-124 and a reduction of GR-α was observed in patient T-cells compared to healthy controls. The authors suggested a novel miR-124-mediated mechanism in the down-regulation of GR-α in patients with critical illness-related corticosteroid insufficiency, that could explain, at least in part, GC resistance in this disease.

Tessel et al\textsuperscript{[87]} have identified and characterized miR-130b as an important down-regulator of GR in GC-resistant multiple myeloma cell line: the overexpression of this miRNA was also associated with a decreased regulation of the downstream GC controlled gene GILZ, suggesting this mechanism as one of the possible causes of resistance to GCs.

**miRNA involved in IBD**

The pathophysiology of IBD is not yet clear, and genetic, epigenetic, infectious and immunological factors seem to play a role. It has been suggested that the gastrointestinal inflammation is the result of an altered activation of the immune system to a luminal factor, such as intestinal flora, in genetically predisposed subjects.

Among the many biological processes regulated by miRNAs, it is now accepted that these small non coding RNAs contribute to the maintenance of immunological homeostasis at mucosal sites\textsuperscript{[88,89]}. The role of miRNAs in the pathogenesis of IBD has been thoroughly considered (see recent reviews\textsuperscript{[32,90,91]}), and it has been suggested that these small non coding RNAs represent an important player in the complex interactions which results in IBD clinical features. Of particular interest is the observation that miRNA expression changes during tissue progres-
tion from normal to inflamed and varies according to the type and evolutionary stage of IBD\cite{99}. Indeed, a number of studies have identified a specific differential expression of miRNAs in IBD and unique miRNA expression profiles for the different subtypes of IBDs, both in human tissues collected by colonoscopic biopsies and in peripheral blood samples, have been demonstrated\cite{100,101}

It has been argued that genetic polymorphisms in miRNAs, as well as in miRNA target genes can affect their regulatory function and, consequently, the expression level of their target mRNAs. Most studies have described an association between SNPs in miRNA genes and human cancers\cite{102,103}, and only recently the association between miRNA related SNPs and the risk of IBD has been examined\cite{104}. Bioinformatic approaches have been used to analyze the association between diseases-linked SNPs, miRNAs and mRNAs: SNP data derived from genome wide association studies that were correlated with miRNA, revealed a CD phenocoding comprising rs11209026, rs7807268, rs254215, rs2542151 in miR-125, rs11805303 in miR-519, and rs6908425 in miR-181\cite{105}. Of interest, miR-181, miR-519 and miR-119 could target mRNAs encoded by genes involved in the importin pathway, whereas miR-181 and miR-125 are potential regulators of components of inflammasome pathway. Both importin and inflammasome are involved also in GC molecular mechanism: importin is a nuclear transport protein responsible for the translocation of the complex GR-GC into the nucleus\cite{106}, and variants in inflammasomes gene have been correlated with steroid resistance in pediatric IBD patients\cite{107}.

An association between rs3746444 in miR-499 and UC susceptibility has been observed in 170 Japanese patients: this SNP may alter the function or expression of miR-499, altering the regulation of target mRNAs related to inflammatory immune responses, and influencing the pathophysiological features of UC\cite{108}. Of particular interest is the observation that the rs3746444 AG genotype was associated also with steroid dependence and refractory phenotype, whereas the rs3746444 AA genotype was inversely related to hospitalization time, steroid dependence, and refractory phenotype. In addition, the rs11614913 TT genotype held a significantly higher risk of refractory phenotype.

CONCLUSION

There is a lot of interest in identifying the role of miRNAs in the modulation of drug response, but studies about this topic are still very limited, and the possible correlation between miRNAs expression and variability in GC response in IBD patients has not yet been extensively examined. Studies about miRNAs and pharmacogenomics may represent a promising investigation topic that could increase the understanding of the pharmacology of steroids in IBDs and possibly in other diseases.

A number of studies have shown that GCs can modify the expression profile of different miRNAs, however, the obtained results have been highly variable. The differences observed can possibly be ascribed to the different tissues or cell lines analysed or different experimental protocols, and to date it is not possible to recognize a specific miRNA pattern regulated by GCs.

miRNA regulation by GCs in IBDs has never been analyzed in clinical prospective studies, in which patients are followed from diagnosis and throughout steroid therapy: the identification of miRNAs differently expressed as a consequence of GC treatment in comparison to diagnosis, could be an important innovative approach. This type of study design will reduce to the minimum the effect of confounding factors and results should be easier to translate into clinical practice.

Moreover, existing studies employ techniques based on the use of reverse transcription quantitative PCR and microarrays, based on the analysis and quantification of already known miRNAs. Using next generation sequencing technologies it should be possible to detect novel, still unrecognised miRNAs, and identify new miRNA isoforms (iso-miRs) as well.

In the future, the increased availability and the reduced costs of RNA profiling should enable the clinicians to stratify patients on specific miRNA biomarkers before starting GC treatment. This will allow the personalization of therapy, avoiding a treatment doomed to failure, increasing efficacy and reducing toxicity.

REFERENCES

1 Friedman S. General principles of medical therapy of inflammatory bowel disease. Gastroenterol Clin North Am 2004; 33: 191-208. viii [PMID: 15177534 DOI: 10.1016/j.gct.2004.02.003]
2 De Iudicibus S, Franca R, Martelossi S, Ventura A, Decorti G. Molecular mechanism of glucocorticoid resistance in inflammatory bowel disease. World J Gastroenterol 2011; 17: 1095-1108 [PMID: 21484144 DOI: 10.3738/wjg.v17.i105]
3 Barnes PJ, Adcock IM. Glucocorticoid resistance in inflammatory diseases. Lancet 2009; 373: 1905-1917 [PMID: 19482216 DOI: 10.1016/S0140-6736(09)6026-3]
4 Barnes PJ. Mechanisms and resistance in glucocorticoid control of inflammation. J Steroid Biochem Mol Biol 2010; 120: 76-85 [PMID: 20188860 DOI: 10.1016/j.jsbmb.2010.02.018]
5 Farrell RJ, Kelleher D. Glucocorticoid resistance in inflammatory bowel disease. J Endocrinol 2003; 178: 339-346 [PMID: 12967327]
6 Guo H, Ingolia NT, Weissman JS, Bartel DP. Mammalian microRNAs predominantly act to decrease target mRNA levels. Nature 2010; 466: 835-840 [PMID: 20703300 DOI: 10.1038/nature09267]
7 Rigoutsos I. New tricks for animal microRNAs: targeting of amino acid coding regions at conserved and nonconserved sites. Cancer Res 2009; 69: 3245-3248 [PMID: 19351814 DOI: 10.1158/0008-5472.CAN-09-0352]
8 Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297 [PMID: 14744438]
9 Kozomara A, Griffiths-Jones S. miRBase: integrating miRNA annotation and deep-sequencing data. Nucleic Acids Res 2011; 39: D152-D157 [PMID: 21037258 DOI: 10.1093/nar/gkr1027]
10 Griffiths-Jones S, Grocock RJ, van Dongen S, Bateman A, Enright AJ, miRBase: microRNA sequences, targets and gene nomenclature. Nucleic Acids Res 2006; 34: D140-D144 [PMID: 16381052 DOI: 10.1093/nar/gkj112]
Oakley RH, Sar M, Cidlowski JA. The human glucocorticoid receptor beta isoform. Expression, biochemical properties, and putative function. *J Biol Chem* 1996; 271: 9550-9559 [PMID: 8621628]

Wu I, Shin SC, Cao Y, Bender IK, Jafari N, Feng G, Lin S, Cidlowski JA, Schleimer RP, Lu NZ. Selective glucocorticoid receptor translational isoforms reveal glucocorticoid-induced apoptotic transcriptomes. *Cell Death Dis* 2013; 4: e453 [PMID: 23301277 DOI: 10.1038/cddis.2012.193]

Lu NZ, Cidlowski JA. Translational regulatory mechanisms generate N-terminal glucocorticoid receptor isoforms with unique transcriptional target genes. *Mol Cell* 2005; 18: 331-342 [PMID: 15866175 DOI: 10.1016/j.molcel.2005.03.025]

Hutchison KA, Scherrer LC, Czar MJ, Ying Y, Sanchez ER, Leach KL, Deibel MR, Pratt WB. FK506 binding to the 56-kilodalton immunophilin (Hsp90) in the glucocorticoid receptor heterocomplex has no effect on receptor folding or function. *Biochemistry* 1993; 32: 3953-3957 [PMID: 7682438]

Pratt WB, Morishima Y, Murphy M, Harrell M. Chaperoning of glucocorticoid receptors. *Handb Exp Pharmacol* 2006; (172): 111-138 [PMID: 16610357]

Gross KL, Lu NZ, Cidlowski JA. Molecular mechanisms regulating glucocorticoid sensitivity and resistance. *Mol Cell Endocrinol* 2009; 300: 7-16 [PMID: 19000736 DOI: 10.1016/j.mce.2008.10.001]

Wikström AC. Glucocorticoid action and novel mechanisms of steroid resistance: role of glucocorticoid receptor-interacting proteins for glucocorticoid responsiveness. *J Endocrinol* 2003; 178: 331-337 [PMID: 12967326]

Qian X, Zhu Y, Xu W, Lin Y. Glucocorticoid receptor and heat shock protein 90 in peripheral blood mononuclear cells from asthmatics. *Chin Med J (Eng)* 2001; 114: 1051-1054 [PMID: 11677765]

Raddatz D, Middel P, Bockemühl M, Benöhr P, Wissmann H, Ramadori G. Glucocorticoid receptor expression in inflammatory bowel disease: evidence for a mucosal down-regulation in steroid-unresponsive ulcerative colitis. *Aliment Pharmacol Ther* 2004; 19: 47-61 [PMID: 14687166]

Matysiak M, Makosa B, Walczak A, Selmaj K. Patients with multiple sclerosis resisted to glucocorticoid therapy: abnormal expression of heat-shock protein 90 in glucocorticoid receptor complex. *Mult Scler* 2008; 14: 919-926 [PMID: 18573821 DOI: 10.1111/j.1352-5508.2008.00666.x]

Charmandari E, Kino T. Chrousos syndrome: a seminal role in pathogenesis of adenral incidentalomas: potential role of reduced sensitivity to glucocorticoids. *Mol Med* 2012; 18: 1456-1465 [PMID: 23196783 DOI: 10.2119/molmed.2012.02626.x]

Ouyang J, Chen P, Jiang T, Chen Y, Li J. Nuclear HSP90 regulates the glucocorticoid responsiveness of PBMCs in patients with idiopathic nephrotic syndrome. *Int Immunopharmacol* 2012; 14: 334-340 [PMID: 22926076 DOI: 10.1016/j.intimp.2012.08.012]

Pemberton LF, Paschal BM. Mechanisms of receptor-mediating nuclear import and nuclear export. *Traffic* 2005; 6: 187-198 [PMID: 15702987 DOI: 10.1111/j.1600-0854.2005.00270.x]

Almawi WY, Melemedjian OK. Molecular mechanisms of glucocorticoid antiproliferative effects: antagonism of transcription factor activity by glucocorticoid receptor. *J Leukoc Biol* 2002; 71: 9-15 [PMID: 11781376]

Meijssing SH, Pufall MA, So AY, Bates DL, Chen L, Yamamoto KR. DNA binding site sequence directs glucocorticoid receptor structure and activity. *Science* 2009; 324: 407-410 [PMID: 19322434 DOI: 10.1126/science.1164265]

Nordeen SK, Suh BJ, Kühnel B, Hutchison CA. Structural determinants of a glucocorticoid receptor recognition element. *Mol Endocrinol* 1990; 4: 1866-1873 [PMID: 1964489]

De Bosscher K, Vanden Berghe W, Vermeulen L, Plaisance S, Boone E, Haegeman G. Glucocorticoids repress NF-kappaB-driven genes by disturbing the interaction of p65 with the basal transcription machinery, irrespective of coactivator levels in the cell. *Proc Natl Acad Sci USA* 2007; 99: 3919-3924 [PMID: 17062063]

Sächke H, Döcke WD, Asadullah K. Mechanisms involved in the side effects of glucocorticoids. *Pharmacol Ther* 2002; 96: 23-43 [PMID: 12441176]

Sächke H, Schottelius A, Döcke WD, Streilke P, Jaroch S, Schmees N, Rehwinkel H, Hennekens H, Asadullah K. Dissociation of transactivation from transrepression by a selective glucocorticoid receptor agonist leads to separation of therapeutic effects from side effects. *Proc Natl Acad Sci USA* 2004; 101: 227-232 [PMID: 14694204 DOI: 10.1073/pnas.0300772101]

Song IH, Gold R, Straub RH, Burmester GR, Buttgereit F. New glucocorticoids on the horizon: repress, don’t activate! *J Rheumatol* 2005; 32: 1199-1207 [PMID: 16041872]

Chen R, Burke TF, Cumberland JE, Brummet M, Beck LA, Casaloro V, Georas SN. Glucocorticoids inhibit calcium- and calcineurin-dependent activation of the human IL-4 promoter. *J Immunol* 2000; 164: 825-832 [PMID: 10623828]

Ing NH. Steroid hormones regulate gene expression post-transcriptionally by altering the stabilities of messenger RNAs. *Bio Reprod* 2005; 72: 1290-1296 [PMID: 15728791 DOI: 10.1095/bioreprod.105.040414]

Croxall JD, van Hal PT, Choudhury Q, Gilroy DW, Flower RJ. Different glucocorticoids vary in their genomic and non-genomic mechanism of action in A549 cells. *Br J Pharmacol* 2002, 135: 511-519 [PMID: 11815387 DOI: 10.1038/sj.bjp.0704474]

Croxall JD, Flower RJ. Lipoicortin 1 mediates dexamethasone-induced growth arrest of the A549 lung adenocarcinoma cell line. *Proc Natl Acad Sci USA* 1992; 89: 3571-3575 [PMID: 1533045]

McConkey DJ, Nicotera P, Hartzell P, Bellomo G, Wylie AH, Ormennis S. Glucocorticoids activate a suicide process in thymocytes through an elevation of cytosolic Ca++ concentration. *Arch Biochem Biophys* 1989; 269: 365-370 [PMID: 2537063 DOI: 10.1016/0003-9866(89)90117-9]

Cohen JJ, Duke RC. Glucocorticoid activation of a calcium-dependent endonuclease in thymocyte nuclei leads to cell death. *J Immunol* 1984; 132: 38-42 [PMID: 6317746]

Sionov RV. MicroRNAs and Glucocorticoid-Induced Apoptosis in Lymphoid Malignancies. *iScience* 2013; 348212 [PMID: 23451463 DOI: 10.1155/2013/348212]

Rainer J, Ploner C, Jesacher S, Ploner A, Eduardoff M, Mansha M, Wasim M, Panzer-Grümayer R, Trajanoski Z, Niederegger H, Kofler R. Glucocorticoid-regulated microRNAs and mirtrons in acute lymphoblastic leukemia. *Arch Biochem Biophys* 2009; 486: 318-328 [PMID: 1964489 DOI: 10.1016/j.abb.2009.05.023]

Molitoris JK, McColl KS, Distelhorst CW. Glucocorticoid-mediated repression of the oncogenic microRNA cluster miR-17–92 contributes to the induction of Bim and initiation of apoptosis. *Mol Endocrinol* 2011; 25: 409-420 [PMID: 21296910 DOI: 10.1210/me.2010-0402]

Harada M, Pokrovskaja-Tamm K, Söderhall S, Heyman M, Grander D, Corcoran M. Involvement of mir17 pathway in glucocorticoid-induced cell death in pediatric acute lymphoblastic leukemia. *Leuk Lymphoma* 2012; 53: 2041-2050 [PMID: 22475310 DOI: 10.3109/10428194.2012.678004]

Yan J, Jiang N, Huang G, Tay JL, Lin B, Bi C, Koh GS, Li Z.
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Tan J, Chung TH, Lu Y, Ariffin H, Kham SK, Yeoh AE, Chng WJ. Deregulated MIR335 that targets MAPK1 is implicated in poor outcome of paediatric acute lymphoblastic leukaemia. Br J Haematol 2013; 163: 93-103 [PMID: 23888996 DOI: 10.1111/bjh.12489]

Smith LK, Shah RR, Cidlowski JA. Glucocorticoids modulate microRNA expression and processing during lymphocyte apoptosis. J Biol Chem 2010; 285: 36698-36708 [PMID: 20847043 DOI: 10.1074/jbc.M110.162123]

Lederose C, Mühlen P, Limbeck E, Schutz S, Weis F, Rink J, Briegel J, Kreth S. Corticosteroid resistance in sepsis is induced by microRNA-124-induced downregulation of glucocorticoid receptor-α. Crit Care Med 2012; 40: 2745-2753 [PMID: 22867681 DOI: 10.1097/CCM.0b013e318258bebc]

Liu L, Walker EA, Kissane S, Khan I, Murray PI, Rauz S, Wallace GR. Gene expression and miRNA levels of human corneal fibroblasts in response to dexamethasone. Invest Ophthalmol Vis Sci 2011; 52: 7282-7288 [PMID: 21662410 DOI: 10.1167/iovs.11-7465]

Lu S, Mukkada VA, Mangray S, Cleveland K, Shillingford N, Schorf C, Brodsky AS, Resnick MB. MicroRNA profiling in mucosal biopsies of eosinophilic esophagitis patients pre and post treatment with steroids and relationship with mRNA targets. PLoS One 2009; 4: e4676 [PMID: 19251514 DOI: 10.1371/journal.pone.0004676]

Williams AE, Larmer-Svensson H, Perry MM, Campbell GA, Herrick VE, Adcock IM, Erjefalt JS, Chung KF, Lindsay MA. microRNA expression profiling in mild asthmatic human airways and effect of corticosteroid therapy. PLoS One 2009; 4: e5889 [PMID: 19521518 DOI: 10.1371/journal.pone.0005889]

Davis TE, Kis-Toth K, Szanto A, Tsokos GC. Glucocorticoids suppress T cell function by up-regulating microRNA-98. Arthritis Rheum 2013; 65: 1882-1890 [PMID: 23575983 DOI: 10.1002/art.37966]

Kertesz M, Iovino N, Unnerstall U, Gau U, Segal E. The role of site accessibility in microRNA target recognition. Nat Genet 2007; 39: 1278-1284 [PMID: 17993677 DOI: 10.1038/ng1235]

Vreugdenhil E, Verissimo CS, Mariman E, Kamporst JH, Barbosa JS, Zweers T, Champagne DL, Schouten T, Meijer Vreugdenhil E. Identification of microRNAs associated with ileal and colonic Crohn’s disease. Inflamm Bowel Dis 2010; 16: 1729-1738 [PMID: 20848482 DOI: 10.1002/ibd.21267]

Hu Z, Chen J, Tian T, Zhou X, Gu H, Xu L, Zeng Y, Miao R, Jin G, Ma H, Chen Y, Shen H. Genetic variants of miRNA sequences and non-small cell lung cancer survival. J Clin Invest 2008; 118: 2600-2608 [PMID: 18521189 DOI: 10.1172/JCI34954]

Hu Z. Insight into microRNA regulation by analyzing the characteristics of their targets in humans. BMC Genomics 2009; 10: 594 [PMID: 20030303 DOI: 10.1186/1471-2164-10-594]

Jazdzewski K, Murray EL, Franssila K, Jarzab B, Schoenberg DR, de la Chapelle A. Common SNP in pre-miR-146a decreases mature miR expression and predisposes to papillary thyroid carcinoma. Proc Natl Acad Sci USA 2008; 105: 2409-2413 [PMID: 18477274 DOI: 10.1073/pnas.0802682105]

Tian T, Shu Y, Chen J, Hu Z, Xu L, Jin G, Liang J, Liu P, Zhou M, Xiao R, Ma H, Chen Y, Shen H. A functional genetic variant in microRNA-196a2 is associated with increased susceptibility of lung cancer in Chinese. Cancer Epidemiol Biomarkers Prev 2009; 18: 1183-1187 [PMID: 19293314 DOI: 10.1183/1538-7445-2009.04.04.0814]

Xu T, Zhu Y, Wei QK, Yuan Y, Zhou F, Ge YY, Yang JR, Su H, Zhuang SM. A functional polymorphism in the miR-146a gene is associated with the risk for hepatocellular carcinoma. Carcinogenesis 2008; 29: 2126-2131 [PMID: 18711148 DOI: 10.1093/carcin/bgn195]

Wang N, Tian QZ, Li Y, Zhou RM, Wang GY. An A/G polymorphism rs3746444 in miR-499 is associated with increased cancer risk: a meta-analysis. Genet Mol Res 2013; 12: 3955-3964 [PMID: 24083577 DOI: 10.4238/2013.03mar.0407]

Gazouli M, Papapantoniou I, Stamatis K, Vaiopoulos A, Zeglinas C, Vassiliou I, Giokas G, Tzathas C. Association study of genetic variants in miRNAs in patients with inflammatory bowel disease: preliminary results. Dig Dis Sci 2013; 58: 2324-2328 [PMID: 23543085 DOI: 10.1007/s10629-013-2640-x]

De Iudicibus S, Stocco G, Martellossi S, Londero M, Ebner E, Pontillo A, Lionetti P, Barabino A, Bartolli F, Ventura A, Decorti G. Genetic predictors of glucocorticoid response in pediatric patients with inflammatory bowel diseases. J Clin Gastroenterol 2011; 45: e1-e7 [PMID: 20697295 DOI: 10.1097/MCG.0b013e3181e8a6f5]

Okubo M, Tahara T, Shibata T, Yamashita H, Nakamura M, Yoshikawa D, Yonemura J, Kamiya Y, Ishizuka T, Nakagawa Y, Nagasaka M, Iwata M, Yamada H, Hirata I, Arissawa T. Association study of common genetic variants in pre-microRNAs in patients with ulcerative colitis. J Clin Immunol 2011; 31: 69-73 [PMID: 20848167 DOI: 10.1007/s10875-010-9461-y]

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90 Coskun M, Bjerrum JT, Seidelin JB, Nielsen OH. MicroRNAs in inflammatory bowel disease—pathogenesis, diagnostics and therapeutics. World J Gastroenterol 2012; 18: 4629-4634 [PMID: 23002331 DOI: 10.3748/wjg.v18.i34.4629]

91 Dalal SR, Kwon JH. The Role of MicroRNA in Inflammatory Bowel Disease. Gastroenterol Hepatol (N Y) 2010; 6: 714-722 [PMID: 21437020]

92 Wu F, Zhang S, Dassopoulos T, Harris ML, Bayless TM, Meulier SJ, Brant SR, Kwon JH. Identification of microRNAs associated with ileal and colonic Crohn’s disease. Inflamm Bowel Dis 2010; 16: 1729-1738 [PMID: 20848482 DOI: 10.1002/ibd.21267]

93 Coskun M, Bjerrum JT, Seidelin JB, Nielsen OH. MicroRNAs in inflammatory bowel disease—pathogenesis, diagnostics and therapeutics. World J Gastroenterol 2012; 18: 4629-4634 [PMID: 23002331 DOI: 10.3748/wjg.v18.i34.4629]

94 Dalal SR, Kwon JH. The Role of MicroRNA in Inflammatory Bowel Disease. Gastroenterol Hepatol (N Y) 2010; 6: 714-722 [PMID: 21437020]

95 Coskun M, Bjerrum JT, Seidelin JB, Nielsen OH. MicroRNAs in inflammatory bowel disease—pathogenesis, diagnostics and therapeutics. World J Gastroenterol 2012; 18: 4629-4634 [PMID: 23002331 DOI: 10.3748/wjg.v18.i34.4629]

96 Dalal SR, Kwon JH. The Role of MicroRNA in Inflammatory Bowel Disease. Gastroenterol Hepatol (N Y) 2010; 6: 714-722 [PMID: 21437020]

97 Coskun M, Bjerrum JT, Seidelin JB, Nielsen OH. MicroRNAs in inflammatory bowel disease—pathogenesis, diagnostics and therapeutics. World J Gastroenterol 2012; 18: 4629-4634 [PMID: 23002331 DOI: 10.3748/wjg.v18.i34.4629]

98 Dalal SR, Kwon JH. The Role of MicroRNA in Inflammatory Bowel Disease. Gastroenterol Hepatol (N Y) 2010; 6: 714-722 [PMID: 21437020]
