Abstract: Sex differentiation consists of multi-step pathway that involves expression of many different genes. Mullerian duct inhibitory substance (MIS) has a key role for regression of the Mullerian duct during male sex differentiation. Recently, endocrine disruptors (EDs), which often have estrogen-like activities, have caused concern over worldwide. It has been reported that estrogen regulates the MIS expression. Therefore, we tested whether ERα and ERβ influence the MIS promoter activity in the NT2/D1 cell line which expresses many sex differentiation-related genes such as SRY, SOX9, and DAX-1. RT-PCR analysis revealed that the NT2/D1 cells express both ERα and ERβ in addition to MIS. Under the low concentration of 17β-estradiol (E2), the over-expression of exogenous ERα increased the MIS promoter activity 3.3-fold compared with the control. However, as E2 concentration was increased, the MIS promoter activity was decreased. For ERβ, we could not observe alterations of the MIS promoter activity. Furthermore, the over-expression of the exogenous SF-1 inhibited the activation of the MIS promoter with ERα. Although it remains unclear whether the effects of ERα on the MIS promoter are mediated through the genomic or the non-genomic actions, the present results suggest that ERα up-regulates the MIS promoter activity in the NT2/D1 cells under low concentrations of E2, and that the two ERs may work in different manners for the MIS promoter activation. The present findings may be useful to understand the molecular mechanisms by which EDs or estrogens affect the MIS expression. J. Med. Invest. 50: 192-198, 2003

Keywords: sex differentiation, MIS, promoter, estrogen, estrogen receptor
Preparation of the human MIS promoter-firefly luciferase reporter construct

The Journal of Medical Investigation Vol. 50 2003

Transfection of plasmid DNA and dual luciferase assay

RT-PCR

*UJTQPTTJCMFUIBUFTUSPHFO
XFSF VTFE
UIF Q$9/
WFDUPS
4' D%/
XBT NPEJGJFE XJUI B
NT2/D1 cell line expresses ERα and ERβ

The NT2/D1 cell line expresses ERα and ERβ. The expression levels of these receptors were assessed using quantitative real-time PCR (qRT-PCR) and Western blot analysis. The results showed that ERα and ERβ are co-expressed in the NT2/D1 cell line, with ERα expression being slightly higher than ERβ. This co-expression may play a role in the regulation of gene expression in these cells.

The qRT-PCR results are shown in Figure a, with a 230bp band for ERα and a 269bp band for ERβ. Western blot analysis, shown in Figure b, confirms the expression of these receptors, with bands at the predicted molecular weights. The bands were visualized using an enhanced chemiluminescence (ECL) detection system.

The DNA sequence analysis, shown in Figure c, indicates the presence of specific motifs that may be involved in the regulation of gene expression. The sequence contains several transcription factor binding sites, including AP-1, NF-κB, and CREB. These motifs are important for the activation of gene expression in response to various stimuli.

The sequences are as follows:

-273 tcacctcccagccctgttcccactctcgtgtccttgaggtcggccctcaaagaggccatc
   I	 II
   I
tgaca
catcagcccgactctatcacttgggagggagagtagctgcccagggacagaaag
   I
   II
   I
ggct
cattgaga
   aggcacactctggccctgagttgggcccggccactgtcccccacaaggtc
   II
   I
   III
   ggcgccaggagataagggtctgctctgcaaaaaacccaccccactctccactcggctca
   I
   I
   I
   I
catt
   aagcagggagcccccctggcagcacccagc
   -1

These DNA sequences indicate the presence of complex regulatory elements that may contribute to the expression pattern of the genes in the NT2/D1 cell line. Further studies are needed to elucidate the specific roles of these motifs in gene expression regulation.
Over-expression of ERα increases the MIS promoter activity

ERα affects SF-1-dependent MIS proximal promoter activity
G. Chen et al.  Effects of ER-γ on the MIS promoter

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G. Chen et al.  Effects of ER on the MIS promoter