Evaluation of the Expression of Isoforms of Stem Cell Factor mRNA in Fetal Tissues and Mononuclear Cells at Different Stages of Human Development

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We studied the expression of isoforms of stem cells factor mRNA forming as a result of alternative splicing. Both isoforms of stem cell factor mRNA forming as a result of alternative splicing were found in different fetal tissues. Changes in the expression of alternative isoforms of stem cell factor in peripheral blood mononuclear cells were demonstrated from the prenatal and neonatal periods to adult organism.

Key Words: *alternative splicing; stem cell factor; gene expression; ontogeny*

Stem cell factor (SCF), an important hemopoietic growth factor, binds and activates c-kit receptor, a representative of tyrosine kinase family and a prooncogen [4]. SCF plays an important role in the regulation of growth and proliferation of hemopoietic stem cells, mast cell precursors, melanocytes, and a key cytokine in processes of sex cell growth and maturation [1,2,10].

SCF exists in soluble and membrane forms [7,13]. The membrane form is synthesized during alternative splicing of *scf* gene mRNA. This process leads to elimination of exon 6 and formation of a shortened transcript. In full-length mRNA, exon 6 is still present and carries a site of proteolytic cleavage, which leads to the formation of a soluble isoform [1]. Recent studies showed that the membrane and soluble forms differ by their functional characteristics [2,9].

The mechanisms of regulation of alternative splicing and tissue—specific expression of SCF are poorly studied.

Here we studied the expression of SCF isoforms in different tissues in the prenatal period and in mononuclear blood cells at different stages of human ontogeny.

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MATERIALS AND METHODS

Tissue samples and blood of fetuses (N=12) from relatively healthy women (mean age 26±3 years), in whom the pregnancy was interrupted by social indications (mean term of gestation 20-22 weeks) were used in the study. Abortion was induced by intraamnial injection of 25 mg enzaptost. The absence of intrauterine infection (herpes, hepatitis B and C, cytomegalovirus, *etc.*) in the fetus was confirmed by bacteriological and histological examination of the abortion material and placenta.

The study also included women in whom the pregnancy eventuated by spontaneous term delivery. Umbilical cord blood (5-7 ml) of healthy full-term newborns (N=13) was taken after cutting the umbilical cord, when the placenta still was in the uterus (*in utero*). To this end, the umbilical vein was drained and self-flowing blood was collected into a sterile tube with 1 ml 0.5 M EDTA. All studies on fetal tissues and fetal/umbilical cord blood were approved by local ethical committee; signed informed consent was obtained from all patients.

Mononuclear cells (MNC) from fetal/umbilical blood samples and from the peripheral blood of conventionally healthy adult donors (*N*=15) were isolated by routine centrifugation in Ficoll-urografin density gradient (ρ=1.082 g/liter) [3]. Total RNA was isolated using the method of phenol extraction [5]. The quality of isolated RNA was evaluated by electrophoresis in 1% agarose gel (Chemapol) prepared on tris-acetate buffer.

For reverse transcription reaction, 1 μg total RNA was denaturated in the presence of universal oligo(dT)16 primer at 72°C for 5 min and transferred on ice. The reaction mixture contained 1 μg total RNA, 0.5 μg oligo(dT)16, 500 μmol each dNTP (Sigma), and 20 U M-MuLV reverse transcriptase (Biosan). The reaction buffer was supplied together with reverse transcriptase and contained 20 mM $MgCl_2$, 20 mM Tris-HCl (pH 8.3 at 25°C), 100 mM KCl, and 1 mM dithiothreitol (DTT). The reaction was carried out in a volume of 20 μl for 1.5 h at 37°C; the enzyme was denaturated for 5 min at 95°C.

Then, PCR with obtained cDNA samples and pairs of primers specific to transcript sequences for soluble and bound SCF isoforms was performed. For improving the sensitivity and specificity of the reaction, nested PCR with the obtained products was performed.

Primers to the sequence of matrix (mRNA) SCF (Ac.NO NM_000899) were synthesized in Biosan Company.

PCR products were analyzed by electrophoresis in 2% agarose gel stained with ethidium bromide (0.5 μg/ml) and then visualized in UV light. Molecular weight of fragments and integral optical density of bands (IOD) were evaluated using a videodensitometer and ImageMaster VDS software (Pharmacia Biotech).

We analyzed total RNA samples isolated from the liver (*n*=7), spleen (*n*=5), thymus (*n*=6), ovaries (*n*=5), testes (*n*= 4), kidney (*n*=5), brain (*n*=7), adrenal glands (*n*=3), thyroid gland (*n*=1), lungs (*n*=1), tooth germ (*n*=3), and skin (*n*=1).

RESULTS

Analysis of SCF expression in samples obtained from one fetus revealed unequal expression of isoforms in different tissues of the same organism. In one case, expression of both isoforms was observed in the liver, while in the kidney of the same fetus only soluble SCF was detected. In other case, pronounced expression of both isoforms in the testes was found, but no expression was detected in tooth germ from the same fetus. In one case, changes in isoform expression profile were noted: membrane isoform mRNA was stably detected in the spleen, only soluble form was present in the thymus, and no expression was found in the liver. This confirms tissue-specific character of regulation of alternative splicing of SCF and probably the dependence of the expression on physiological state of the fetus.

However, analysis of the data presented in Table 1 and obtained during examination of all collected samples without considering their source showed that expression of both mRNA SCF isoforms was detected in all studied tissues except for thyroid gland and tooth germs, which attests to polymorphism of SCF-producing cells in these tissues and pleiotropic regulatory influence of this cytokine during the prenatal period.

The above data attest to heterogeneity of SCF expression not only in tissues of the same organism, but also in different samples of the same tissue. It is known that gestation weeks 20-22, apart from active growth, differentiation, and maturation of cells, are characterized by transition from liver to bone marrow hemopoiesis and immunopoiesis [11]. Unstable expression of this or that isoforms in the thymus, spleen, and liver can also be explained by these processes.

SCF regulates activity of sex cells and plays an important role in spermatogenesis and oogenesis [8]. Therefore, it was very important to study alternative splicing of SCF mRNA in the testes and ovaries. It was found that both isoforms are equally presented in these tissues.

Since SCF plays an important role in the maintenance of proliferative activity of stem cells, it was interesting to evaluate the presence of SCF isoforms in tooth germs, because these structures are known to be characterized by high proliferative activity of stem

TABLE 1. Incidence of Splicing Isoforms of Human SCF in Fetal Tissues on Gestation Weeks 20-22

Fetal tissues	SCF soluble	SCF membrane
Liver	5/7	4/7
Thymus	4/6	2/6
Spleen	2/5	2/5
Ovaries	2/5	2/5
Testes	3/4	3/4
Kidney	3/5	2/5
Adrenal glands	3/3	3/3
Brain	5/7	5/7
Tooth germs	1/3	0/3
Thyroid gland	0/1	0/1
Lungs	1/1	0/1

Note. Here and in Table 2: numerator: number of detected positive samples, denominator: total number of samples.

TABLE 2. Incidence of Alternative Transcripts of Human SCF in Blood MNC at Different Stage of Ontogeny

SCF isoforms	MNC of fetal blood on gestation weeks 20-22 (*n*=5)	MNC of umbilical cord blood from newborns (*n*=13)	MNC of peripheral blood from adults (*n*=15)
SCF soluble	5/5	3/13	0/15
SCF membrane	5/5	5/13	0/15

cells [6,12]. However, expression of soluble isoform of SCF mRNA was detected in only one of three examined samples. This can be explained by the fact that the expression intensity also varies during intrauterine development and depends on gestation term and by more strict tissue specificity of alternative splicing in this compartment. The samples were isolated from fetuses differing by the gestation terms by 2-3 weeks; more precise determination of the gestation term was impossible.

At the next stage of the experiment we studied changes in the expression at different developmental stage. We studied the presence of SCF mRNA isoforms in mononuclear cells from fetal blood on weeks 20-22 of intrauterine development, umbilical cord blood of newborns, and peripheral blood of adult donors. Pronounced expression of both isoforms was detected in fetal MNC, while no expression was detected in MNC from adults. Positive expression of mRNA SCF was detected in 5 of 13 samples of umbilical cord blood; it should be noted that two samples contained only the fragment corresponding to membrane isoform (Table 2).

The presence of SCF mRNA isoforms in the blood during the antenatal and neonatal periods can be explained by migration of SCF-producing cells with the blood to the red bone marrow, the major organ of hemopoiesis and hemopoiesis, and to organs characterized by active proliferation of tissue stem cells, *e.g*. testes, skin, mucosae. Unstable picture from one sample to another can be explained by individual peculiarities of newborn organism, its maturity, and term of delivery.

The predominance of membrane isoform during the neonatal period is related to functional peculiarities of migrating cells and attests to different biological role of these isoforms. Experiments on mice prove the important role of membrane isoform in erythropoiesis, regulation of proliferation and activity of melanocytes and tissue mast cells [2]. It can be hypothesized that predominance of cells expressing this isoform attests to more active migration of cells regulating erythropoiesis, because this period is characterized by transition from fetal to neonatal erythropoiesis. The role of membrane isoform in these processes is to be evaluated.

Thus, our experiments demonstrated inhomogeneous tissue-specific expression of SCF mRNA isoforms in the organism and its abundance in many tissues during the prenatal period of human development.

The incidence and spectrum of splicing variants in human blood mononuclear cells depend on the developmental stage and are most pronounced during intrauterine development and in newborns. During the neonatal period, expression of membrane isoform of SCF mRNA predominates.

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