Telomerase Activity in Different Tissues of Zebrafish (*Danio rerio*)

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Geliş Tarihi (Received): 21.07.2014, Kabul Tarihi (Accepted): 15.10.2014

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ABSTRACT

Zebrafish (*Danio rerio*) is a powerful model for aging, cancer, developmental biology and some aging-related diseases. It is critical to investigate complementary vertebrate models to understand telomere and telomerase function in biological systems. One of the best model organisms for telomerase studies is zebrafish. Zebrafish have different telomerase activity in different tissues. These differences may depend on the tissue’s function or regeneration capacity of organs. The aim of this study is to compare the telomerase activity of liver, heart and spleen tissues in juvenile and adult zebrafish. RTA (relative telomerase activity) values of all tissues were different from each other statistically in the adult group (6 months of age). In the juvenile group (2 months of age) there is no difference between heart and spleen values but the other groups are statistically different from each other.

**Key Words:** Zebrafish (*Danio rerio*), telomerase, spleen, heart, liver

ÖZET


**Anahtar Kelimeler:** Zebra balığı (*Danio rerio*), telomerase, dalak, kalp, karaciğer
INTRODUCTION

Telomeres are specialized functional complexes that protect the ends of eukaryotic chromosomes. The essential telomeric DNA sequences at each end of the eukaryotic linear chromosomes are, in most species, tandem repeats of a short sequence unit (Blackburn, 2001). Greider and Blackburn identified a telomere terminal transferase involved in the addition of telomeric repeats necessary for the replication of chromosome ends in eukaryotes (Greider and Blackburn, 1985). Telomerase adds multiple copies of this DNA unit to the terminal portion of one strand of the repeat tract (Blackburn, 2001).

Telomerase is a very important enzyme for aging process and carcinogenesis. Primary human cells exhibited limited replicative potential but the cancer lines divided indefinitely with passage in culture (Artandi and DePinho, 2010). To grow indefinitely, human cancer cells must counteract the progressive loss of telomeric DNA that universally accompanies cell division (Shay et al., 2012). This immortality is a result of telomerase activity mainly. Telomerase is expressed in more than 85% of cancer cells (Buseman et al., 2012), but the telomere length can be maintained in the absence of telomerase. It was deduced that one or more alternative telomerase-independent mechanisms exist in human cells (Shay et al., 2012).

Telomerase shortening may cause aging and death. Some evidence suggests that the progressive loss of telomeric repeats of chromosomes may function as an important timing mechanism during the aging process. Numerous epidemiological studies show that shorter telomeres in humans are associated with many age related diseases (Boccardi and Paolisso, 2014). Telomerase gene therapy in adult and old mice delays aging and increases longevity (de Jesus et al., 2012; de Jesus and Blasco, 2013).

Zebrafish (Danio rerio) has been developed as a powerful model for aging, cancer, developmental biology and some aging-related diseases (Kishi et al., 2003). It is critical to investigate complementary vertebrate models to understand telomere and telomerase function in biological systems. Contrary to the inbred laboratory mouse, zebrafish have heterogeneous telomeres of human-like length and shorten with age. Like humans, telomerase expression in zebrafish somatic cells is not sufficient to prevent telomere shortening (Henriques et al., 2013).

Different tissues have different telomerase activity. Zebrafish also have different telomerase activity in different tissues. These differences may depend on the tissue’s function or regeneration capacity of organs. There is little information about telomerase activity differences in zebrafish tissues in the literature. The aim of this study is to compare the telomerase activity of liver, heart and spleen tissues in juvenile and adult zebrafish. This data will provide a source for zebrafish researchers who study cancer, aging and regeneration in particular.

MATERIALS AND METHODS

Maintenance of Zebrafish

Zebrafish (Danio rerio) were maintained at 24±2°C with a light/dark cycle of 14:10 hours and were fed dry flake food. The fish were anesthetized with ice before organs were excised. Thirty zebrafishes were used for this research as 15 adults and 15 juveniles. All zebrafish applications were approved by the Ethical Committee of the Mehmet Akif Ersoy University (93773921-65).

Telomerase Assay

Telomerase activities were measured using the Roche TeloTAGGG Telomerase PCR ELISA kit according to manufacturer’s instructions. This kit allows highly specific amplification of telomerase-mediated elongation products combined with non-radioactive detection following an ELISA protocol. Relative Telomerase Activity (RTA) values were calculated for mg/ml of protein. Protein values were determined by the Bradford method (Bradford Reagent SIGMA B 6916). Minitab Release 13.0 statistical software was used for analysis. The results were estimated with the Mann-Whitney Test.

RESULTS

RTA values of all tissues were different from each other statistically in the Adult group (6 months of age) (p<0.05). In the Juvenile group (2 months of age) there is no difference between heart and spleen values but the other groups are statistically different from each other (p<0.05) (Table 1 and Figure 1).

Table 1. Relative Telomerase Activity (RTA) ± standard error (SE) values of tissues.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Relative Telomerase Activity (RTA) ± SE</th>
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<tbody>
<tr>
<td>Heart (6 months)</td>
<td>0.301 ± 0.058</td>
</tr>
<tr>
<td>Spleen (6 months)</td>
<td>1.122 ± 0.261</td>
</tr>
<tr>
<td>Liver (6 months)</td>
<td>0.131 ± 0.050</td>
</tr>
<tr>
<td>Heart (2 months)</td>
<td>1.132 ± 0.507</td>
</tr>
<tr>
<td>Spleen (2 months)</td>
<td>0.572 ± 0.206</td>
</tr>
<tr>
<td>Liver (2 months)</td>
<td>0.130 ± 0.032</td>
</tr>
</tbody>
</table>
Expression of telomerase in aquatic animals is likely not related to longevity but to their ability to regenerate injured tissue (Elmore et al., 2008). Although human organs have a limited ability to heal and regenerate damaged or lost tissue, the zebrafish retains remarkable regenerative abilities in most tissues. Moreover, the zebrafish has constitutively abundant telomerase activity in somatic tissues from embryos to adults (Anchelin et al., 2011). According to Lund and colleagues (2009), telomere lengths are similar in Zebrafish liver, heart, kidney, intestine and gill tissues (Lund et al., 2009). Telomerase activity present in the zebrafish organs may maintain the same telomere lengths.

We detected highest telomerase activity in the adult spleen. Hematopoietic tissue is located in the stroma of the spleen and the interstitium of the kidney in Zebrafish (Menke et al., 2011). High telomerase activity in the spleen tissue may depend on the hematopoietic functions of the organ. Telomerase activity was higher in adult spleen tissue than juvenile. Anchelin and colleagues found that both telomerase expression and telomere length increased from embryo to adulthood stages, but they drastically declined in aged fish despite the telomerase activity detected in different tissues of old fish (Anchelin et al., 2011).

The teleost liver plays an important role in the metabolic homeostasis of the body (Menke et al., 2011). Zebrafish have high regeneration capacity in the liver (Sadler et al., 2007; Kan et al., 2009). We measured very similar RTA values in juvenile and adult livers. However the zebrafish liver had the lowest mean RTA values compared to the spleen and the heart.

According to our research, both juvenile and adult fish have high telomerase activity in heart tissue. In another study, 20% of the heart tissue of zebrafish was surgically removed by Poss and colleagues (2002) and they observed full regeneration of the heart within 60 days. Some other studies also confirm the regeneration ability of the zebrafish heart (Kikuchi, 2014; Major and Poss, 2007). High telomerase activity in the zebrafish heart may be connected with the regeneration ability. Investigation of the regeneration ability of the zebrafish heart is important to cure some human heart diseases like myocardial infarction.

Telomerase enzyme is especially important for cancer, aging and regeneration researches. One of the best model organisms for telomerase studies is the zebrafish, but there is limited information in the literature for telomerase activity in different tissues of the zebrafish. Current research contributes to the scientific literature in that respect. This study is also important in terms of telomerase activity evaluated in adult and juvenile fish.

ACKNOWLEDGEMENTS

This study was supported by the Mehmet Akif Ersoy University Scientific Research Projects Unit with a project number of 0202-YL-13.

REFERENCES


