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ORIGINAL ARTICLE

Male Health

Testicular germ cell tumors type 2 have high RNA expression of *LDHB*, the gene for lactate dehydrogenase subunit B

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This study analyzed RNA expression of genes for three serum tumor markers, alpha fetoprotein (AFP), human chorionic gonadotropin (hCG), and lactate dehydrogenase (LDH), in patients with testicular germ cell tumors (TGCT) type 2. The gene *AFP* encodes AFP, the gene for chorionic gonadotropin beta polypeptide 5 (*CGB5*) encodes a major part of the specific beta subunit of hCG, and the genes for LDH subunit A (*LDHA*), LDH subunit B (*LDHB*), and LDH subunit C (*LDHC*) encode three different subunits of LDH. *LDHB* encodes the LDHB subunit present as a tetramer in LDH isoenzyme 1 (LDH-1). We examined three datasets with 203 samples of normal testis tissue (NT) and TGCT type 2. Yolk sac tumor (YST) expressed RNA of *AFP* fourteen thousand times higher than seminoma (SE), embryonal carcinoma (EC), and teratoma (TER) combined ($P = 0.00015$). In the second microarray, choriocarcinoma (CC) expressed RNA of *CGB5* ten times higher than other histologic types of TGCT combined. EC expressed RNA of *LDHB* twice higher than SE, YST and TER combined ($P = 0.000041$). EC expressed RNA of *LDHB* higher than that YST expressed RNA of *AFP* and that CC expressed RNA of *CGB5*. In conclusion, TGCT type 2 expressed RNA of *LDHB* markedly higher than the RNA of 23 other candidate genes for TGCT type 2.

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INTRODUCTION

Current histopathologic classifications of testicular germ cell tumors (TGCT) type 2 of young adults have two main groups, seminoma (SE) and nonseminomatous germ cell tumors (NSGCT).[1] The TNM (T for primary tumor, N for regional lymph node metastases, M for distant metastases) classification includes three serum tumor markers: alpha fetoprotein (S-AFP), human chorionic gonadotropin (S-hCG), and lactate dehydrogenase (S-LDH, Enzyme Committee 1.1.1.27).[2]

Germ cell neoplasia *in situ* (GCNIS) is precursor for all histologies of TGCT.[1,3] NSGCT includes an undifferentiated histologic type (embryonal carcinoma [EC]), and three differentiated histologic types (teratoma [TER], yolk sac tumor [YST], and choriocarcinoma [CC]).[1] In TGCT, YST elements express AFP, and CC elements express hCG.[4] Hence, tumor markers in tumor lesions are background for raised serum tumor markers in patients with TGCT type 2. Patients with undifferentiated histologic types, SE and EC, often have a raised S-LDH.[5]

The TNM classification includes the serum tumor markers because they had prognostic significance in the International Germ Cell Consensus Classification Group (IGCCCG) study.[6] The study stratified patients with metastatic TGCT in good-, intermediate-, and poor-risk groups. International guidelines follow the TNM classification.[7] The serum tumor markers have discordant levels.[8,9] For instance, 40% of patients with metastatic SE have elevated S-LDH and normal S-AFP and S-hCG.[5]

A recent cohort study including all Danish patients with TGCT type 2 from the modern era using a combination chemotherapy regimen with bleomycin, etoposide, and platinum (BEP) confirmed the prognostic significance of the IGCCCG classification.[10] Another study showed that the serum tumor markers separated patients with intermediate risk into two groups: one had a prognosis like that of patients with good risk and another had a prognosis like that of the patients with poor risk.[11]

Two other studies showed that patients with poor risk who had limited decline of an initially raised S-AFP and S-hCG after the first cycles of BEP obtained an improvement of the overall survival if the patients were switched to a more aggressive form for chemotherapy than BEP.[12,13]

The gene *AFP* encodes AFP. hCG is characterized by a specific beta subunit, and six genes including chorionic gonadotropin beta polypeptide 5 (*CGB5*) encode the beta subunit of hCG. LDH consists of five LDH isoenzymes, and each LDH isoenzyme consists of four LDH subunits, either LDHA or LDHB or both.[14] LDH

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isoenzyme 1 (LDH-1) is a tetramer of four LDHB subunits, and LDH isoenzyme 5 (LDH-5) is a tetramer of four LDHA subunits. Adult men have an additional LDH isoenzyme that is specific for testicular germ cells, LDH-X,[15] which is a tetramer of four LDHC subunits. *LDHA, LDHB,* and *LDHC* encode the three LDH subunits of all LDH isoenzymes.

A recent meta-analysis reported how the histologic types of TGCT type 2 expressed RNA of 24 candidate genes for TGCT type 2 in three datasets.[16] Complementarily, our present study aimed to elucidate whether the histologic types of TGCT type 2 differ in RNA expressions of *AFP, CGB5, LDHA, LDHB* and *LDHC,* and to compare RNA expressions of genes for the serum tumor markers with RNA expressions of the 19 previously evaluated candidate genes.

MATERIALS AND METHODS

Materials

The present study evaluated three public available datasets of RNA expression of genes in TGCT type 2.[17–19] The principal investigators for two of the datasets informed about the RNA expressions of 24 candidate genes in tissue samples of normal testis (NT) and TGCT type 2 from the individual patients. Thereby, the study included datasets of two microarray studies and an RNA dataset of a sequencing study.

The first microarray dataset was based on a German study, originally published in 2005.[17] It included three samples of NT and 40 samples of SE. The study used Affymetrix HG-1195A version 2 (ThermoFisher Scientific, Waltham, MA, USA) to analyze RNA expression. The dataset is deposited at Gene Expression Omnibus (GEO) under the accession number GSE 8607 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE8607).

The second dataset was based on a Norwegian microarray study originally published in 2005.[18] It included three samples of NT, three samples of GCNIS, three samples of SE, five samples of EC, four samples of YST, four samples of TER, and one sample of CC. The study used Agilent Whole Human Genome 1A oligo microarray (Agilent Technologies, Santa Clara, CA, USA) to analyze RNA expression of genes.

Agilent's Human 1A Oligo Microarray (version 2) includes over 17 000 60-mer oligonucleotide probes, sourced from the Incyte Foundation Database, and designed to span conserved exons across the transcripts of the targeted full-length genes. The second microarray dataset reported cDNA expression $\log_2$ transformed relative to a reference standard of ten human cell lines.

The dataset of the second microarray is deposited at GEO under the accession number GSE 1818 (https:www.ncbi.nml.nih.gov/geo/query/acc.gi?acc=GSE1818).

The third dataset is part of the Cancer Genome Atlas (TCGA) project, originally published regarding the subgroup of patients with TGCT type 2 in 2018.[19] The dataset included 72 samples of SE, 27 samples of EC, 13 samples of YST, and 16 samples of TER. The RNA expression was determined using RNA sequencing. The dataset is deposited at TCGA project available from https://portal.gdc.cancer.gov/legacy-archive/.

Selection of genes

Before the study was started, we determined to analyze 24 candidate genes for TGCT type 2: five genes for the serum tumor markers and 19 other genes. These candidate genes included six genes for pluripotency, Kruppel-like factor 4 (*KLF4*), LIN28 homolog A (*LIN28A*), MYC oncogene (*MYC*), homeobox transcription factor NANOG (*NANOG*), POU domain class 5, transcription factor 1 (*POU5F1*), and SRY-box 17 (*SOX17*), that were among the genes with the highest RNA expression in GCNIS.[20]

The candidate genes also included protooncogenes that may be upregulated in TGCT type 2, such as cyclin D2 (*CCND2*),[21] MDM2 protooncogene (*MDM2*),[22] *MYC*,[23] v-myc avian myelocytomatosis viral-related oncogene, neuroblastoma-derived (*NMYC*), pituitary tumor-transforming gene 1 (*PTTG1*),[24] and cyclin E1 (*CCNE1*).

The candidate genes also included tumor suppressor genes that may be downregulated in TGCT type 2, such as cyclin-dependent kinase inhibitor 1A (*CDKN1A*), cyclin-dependent kinase inhibitor 2C (*CDKN2C*),[25] phosphatase and tensin homolog (*PTEN*),[26] and retinoblastoma corepressor 1 (*RB1*).[27] The candidate genes also included preferentially expressed antigen in melanoma (*PRAME*), a biomarker that is highly expressed in SE,[28] and DNA methyltransferase 3B (*DNMT3B*), a biomarker that is highly expressed in EC. The candidate genes also included forkhead box D (*FOXOD*).

Definitions and methods

A gene was defined to express RNA significantly in a histologic type of TGCT type 2 in the second microarray if the median RNA expression of the gene in the histologic type was at least twice that of NT. A gene was defined to express RNA insignificantly in TGCT type 2, if NT and TGCT type 2 in the second microarray did not express RNA of the gene significantly different. A gene was defined as a genetic signature of a histologic type of TGCT type 2 if only the single histologic type characteristically and highly expressed RNA of the gene.

Our study summarized RNA expressions of the candidate genes in samples of NT and the histologic types of TGCT type 2. We evaluated whether NT and the histologic types of TGCT type 2 expressed RNA of the candidate genes differently and whether undifferentiated histologic types of TGCT type 2, SE and EC, and differentiated histologic types of NSGCT expressed RNA of the candidate genes differently. Further, we examined whether TGCT type 2 expressed RNA of genes for the serum tumor markers in the tumor lesions different from RNA expression of the 19 comparator genes.

We did not substitute missing data regarding RNA expression of a gene in the datasets. The null hypothesis of the study was that all TGCT type 2 only expressed RNA of the candidate genes insignificantly. Hence, we gave priority to comparisons between single histologic types or groups of histologic types that differed significantly in RNA expression of the genes for the serum tumor markers.

Statistical analyses

We had focus on the median RNA expression of the genes as we summarized the RNA expression of the genes and analyzed whether the histologic types expressed RNA of the genes differently, using Kruskal–Wallis tests. We calculated a meta-analytic *P* value using the Fisher's test as we analyzed the three datasets regarding RNA expressions of genes for the serum tumor markers.[29]

We carried out the statistically two-tailed analyses and considered $P < 0.05$ as statistically significant. The statistical analyses were carried out using Stata version 14.2 (Stata Corp, College Station, TX, USA).

RESULTS

Genes for the serum tumor markers

Our study included three datasets with 203 samples of NT and TGCT type 2.[17–19] **Table 1** shows clinical characteristics of the patients with TGCT type 2 in the TCGA study.[19] Overall, our study evaluated six samples of NT, three samples of GCNIS, 115 samples of SE, and 79 samples of NSGCT. In the three datasets, the RNA expression of the

five genes for the serum tumor markers differed concordantly between the histologic types.

YST was associated with *AFP* (**Figure 1**). In the first microarray, NT and SE did not differ significantly regarding RNA expression of *AFP* (**Figure 1a**). In the second microarray, YST expressed RNA of *AFP* five hundred times higher than NT (94 *vs* 0.17, $P = 0.06$, Kruskal–Wallis test, **Figure 1b**). In the TCGA dataset, YST expressed RNA of *AFP* fourteen thousand times higher than SE, EC, and TER combined (median: 12 883.1 *vs* 0.9, $P = 0.0001$, Kruskal–Wallis test, **Figure 1c**).

Especially YST had a high RNA expression of *AFP*. Some TER had a relatively high RNA expression of *AFP*, likely to be due to undiagnosed YST elements within the TER. Hence, we consider *AFP* to be a genetic signature of YST.

CC was associated with *CGB5* (**Figure 2**). In the first microarray, SE had slightly higher RNA expression of *CGB5* than NT (**Figure 2a**). In the second microarray, CC expressed RNA of *CGB5* nine times higher than NT, but the difference was not statistically significant (9.0 *vs* 1.0, $P = 0.15$, Kruskal–Wallis test, **Figure 2b**). CC expressed RNA of *CGB5* ten times higher than the other histologies of TGCT combined, but the difference was not statistically significant (9.0 *vs* 0.9, $P = 0.10$, Kruskal–Wallis test).

In the first microarray, SE overall had a slightly raised RNA expression of *CGB5* that may be due to the presence of syncytiotrophoblastic cells in some of the SE. In the second microarray, only CC had a high RNA expression of *CGB5*. Hence, we consider *CGB5* to be a genetic signature of CC.

EC was associated with *LDHB* (**Figure 3**). In the first microarray, SE had significantly higher RNA expression of *LDHB* than NT ($P = 0.002$, Kruskal–Wallis test, **Figure 3a**). In the second microarray, EC expressed RNA of *LDHB* six times higher than NT (2.4 *vs* 0.36, $P = 0.025$, Kruskal–Wallis test, **Figure 3b**). In the TCGA dataset, EC expressed RNA of *LDHB* twice higher than SE, YST, and TER combined (median: 45 154 *vs* 18 708, $P = 0.0001$, Kruskal–Wallis test, **Figure 3c**).

In the first microarray, NT and SE did not differ significantly in RNA expression of *LDHA* ($P = 0.28$, Kruskal–Wallis test). In the second microarray, EC expressed RNA of *LDHA* twice higher than NT, but the difference was not statistically significant (1.7 *vs* 0.82, $P = 0.25$, Kruskal–Wallis test). In the TCGA dataset, SE and EC combined expressed RNA of *LDHA* twice higher than YST and TER combined, but the difference was not statistically significant (median: 19 977 *vs* 6897, $P = 0.06$, Kruskal–Wallis test).

In the first microarray, NT expressed RNA of *LDHC* significantly higher than SE ($P = 0.004$, Kruskal–Wallis test). In the second microarray, NT expressed RNA of *LDHC* 53 times higher than SE (median: 53 *vs* 1.0, $P = 0.0495$, Kruskal–Wallis test). Both NT and GCNIS had markedly higher RNA expression of *LDHC* than SE, EC, TER, and YST combined.

Some GCNIS had a relatively high RNA expression of *LDHC* that is likely to be due to undiagnosed NT elements in the GCNIS. Hence, we consider *LDHC* to be a genetic signature of NT.

In the TCGA dataset, *AFP* and *LDHB* were among the genes that had the highest RNA expression in YST and EC, respectively. EC expressed RNA of *LDHB* higher than RNA of *LDHA*. Importantly, EC had a higher median and maximum RNA expression of *LDHB* than that YST had of *AFP*.

Comparative genes

In the previous meta-analysis of the three datasets, EC relatively highly expressed RNA of *LIN28A, CCND2,* and *NANOG*.[16] Nevertheless in the TCGA dataset in our present study, EC expressed RNA of *LDHB* higher than RNA of ten significant comparative genes (**Figure 4**).

Table 1: Clinical characteristics of patients in the cancer genomic atlas project study

Characteristics	*Values*
Age at diagnosis (year), median (range)	31 (14–66)
Stage of TGCT (*n*)	
I	14
IA	27
IB	10
IS	42
II	4
IIA	11
IIB	1
IIC	1
III	1
IIIA	1
IIIB	8
IIIC	5
Preorchiectomy S-AFP (U ml^{-1}), median (range)	5.4 (0.9–37 822)

S-AFP: serum alpha fetoprotein; TGCT: testicular germ cell tumor

Table 2: Meta-analysis of the three datasets of histologic types of testicular germ cell tumor type 2 regarding *P* values for RNA expression of genes for the serum tumor markers

Gene	*Comparison of histologic types*	*Number of included datasets*	*P values in the meta-analyses of the datasets*
AFP	Y *vs* SET	2	0.00015
LDHB	N *vs* S	2	0.00015
LDHB	E *vs* SYT	2	0.000041
LDHA	N *vs* S	2	0.23
LDHA	E *vs* YT	2	0.00021
LDHC	N *vs* S	2	0.0019

AFP: gene for alpha fetoprotein; *LDHA*: gene for lactate dehydrogenase subunit A; *LDHB*: gene for lactate dehydrogenase subunit B; *LDHC*: gene for lactate dehydrogenase subunit C; Y: yolk sac tumor; E: embryonal carcinoma; N: normal testis; S: seminoma; T: teratoma; SET: seminoma, embryonal carcinoma and teratoma combined; SYT: seminoma, yolk sac tumor and teratoma combined; YT: yolk sac tumor and teratoma combined.

Meta-analysis of RNA expression in the histologic types of genes for the serum tumor markers

Meta-analyses of the three datasets that combined the *P* values for RNA expression of genes for the serum tumor markers in histologic types are shown in **Table 2**. YST expressed RNA for *AFP* significantly higher than SE, EC, and TER combined. SE expressed RNA of *LDHB* significantly higher than NT, whereas SE did not express RNA of *LDHA* significantly higher than NT.

DISCUSSION

The study is an *in silico* investigation that increases the knowledge regarding the background for the three serum tumor markers of TGCT type 2. The study demonstrated that EC expressed RNA of *LDHB* markedly higher than RNA of 23 other candidate genes for TGCT type 2. The findings explain why many patients with SE and EC have a raised S-LDH, whereas mainly patients with elements of YST and CC in NSCGT have raised S-AFP and raised S-hCG. Our *LDHB* findings link a high S-LDH and a high S-LDH-1 in patients with TGCT type 2 to a high copy number of the short arm of chromosome 12 (12p), in the malignant germ cells, often in the form of a characteristic isochromosome, i(12p).[30,31]

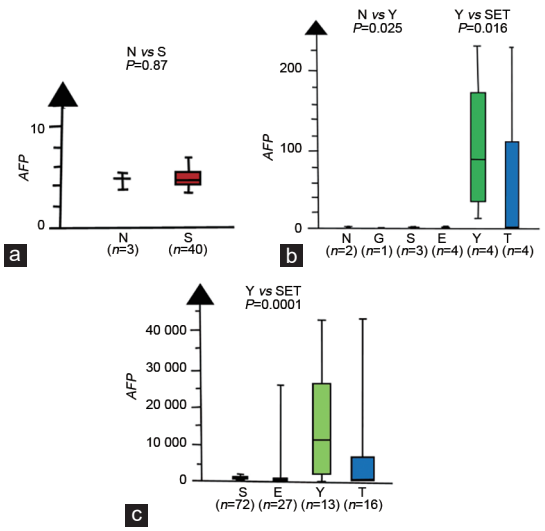

Figure 1: RNA expression of *AFP* in three datasets shows that yolk sac tumor (Y) had higher RNA expression than germ cell neoplasia *in situ* (G), seminoma (S), embryonal carcinoma (E), and teratoma (T). N denotes normal testis. (**a**) The first microarray, (**b**) the second microarray, and (**c**) the TGCA dataset. The boxes show the median and the lower and upper quartiles and the whiskers show the full ranges. The numbers of samples with the respective histologic type in the three datasets are shown. Y *vs* SET is comparison of the RNA expression of *AFP* between Y and S, E, and T combined. *AFP*: the gene for alpha fetoprotein; TGCA: the Cancer Genomic Atlas study.

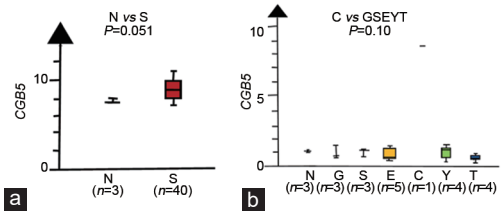

Figure 2: RNA expression of *CGB5* in two microarray datasets. (**a**) The first microarrays show the RNA expression of the gene for chorionic gonadotropin beta polypeptide 5 (*CGB5*) was similar between normal testis (N), and seminoma (S). (**b**) The second microarray shows that choriocarcinoma (C) had a markedly higher RNA expression of *CGB5* than germ cell neoplasia *in situ* (G), S, embryonal carcinoma (E), yolk sac tumor (Y), and teratoma (T) combined (GSEYT).

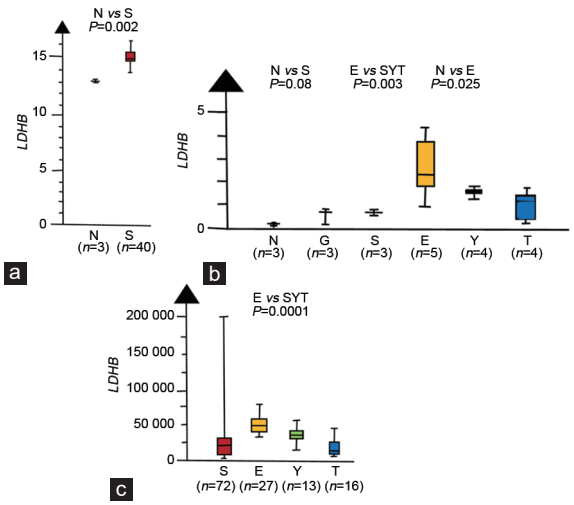

Figure 3: RNA expression of *LDHB* in the three datasets shows that seminoma had higher RNA expression than normal testis (N), and that embryonal carcinoma (E) had higher RNA expression than seminoma (S), yolk sac tumor (Y), and teratoma (T) combined (SYT). (**a**) The first microarray, (**b**) the second microarray, (**c**) the Cancer Genomic Atlas (TGCA) dataset. *LDHB*: gene for lactate dehydrogenase subunit B.

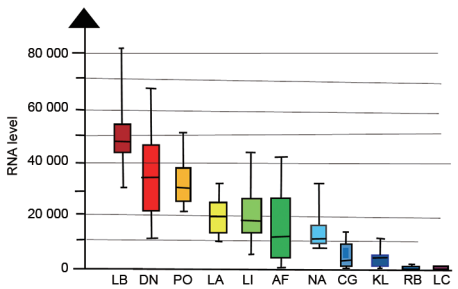

Figure 4: In the Cancer Genomic Atlas (TGCA) dataset, EC expressed RNA of *LDHB* (LB) higher than the RNA of *DNMT3B* (DN), *POU5F1* (PO), *LDHA* (LA), *LIN28* (LI), *AFP* (AF), *NANOG* (NA), *CGB5* (CG), *KLF4* (KL), *RB1* (RB), and *LDHC* (LC). *AFP*: gene for alpha fetoprotein; *LDHA*: gene for lactate dehydrogenase subunit A; *LDHB*: gene for lactate dehydrogenase subunit B; *LDHC*: gene for lactate dehydrogenase subunit C; *DNMT3B*: DNA methyltransferase 3B; *POU5F1*: POU domain class 5, transcription factor 1; *NANOG*: homeobox transcription factor NANOG; *CGB5*: chorionic gonadotropin beta polypeptide 5; *KLF4*: Kruppel-like factor 4; *RB1*: retinoblastoma corepressor 1.

The gene loci for *AFP* (4q13.3) and *CGB5* (19q13.32) are not located on 12p. YST does not have an increased copy number of chromosome 4q, and CC does not have an increased copy number of chromosome 19q.[31] However, the two histologic types of differentiated NSGCT highly expressed RNA of specific genes for AFP and hCG. YST highly expressed RNA of *AFP* and CC highly expressed RNA of *CGB5*. Thus, YST upregulates *AFP* and CC upregulates *CGB5* at the step of transcription of DNA of the genes to RNA. In a previous study of patients with metastatic TGCT type 2 treated with a combination regimen of vinblastine and bleomycin, AFP in the primary tumor lesions was insignificant, whereas hCG in the primary TGCT lesions was a significant prognostic factor.[32] Thus, the prognostic impact of a raised S-AFP in patients with TGCT type 2 might mainly reflect a large volume of AFP-avid elements. In contrast, the prognostic role of S-hCG may in part be due to CC elements irrespective of the extent of the CC elements in NSGCT.

Further, in another study of patients with metastatic TGCT type 2 treated with a regimen of platin, vinblastine, and bleomycin (PVB), both S-LDH and total tumor volume were significant prognostic factors.[33]

Copy number of 12p and RNA expression of *LDHB* in TGCT type 2 lesions wereassociated with the levels of S-LDH/S-LDH-1 in previous studies.[34,35] In patients with TGCT type 2, the product of the overall tumor mass and the copy number of 12p in malignant germ cells in cytogenetic analyses of TGCT type 2 correlated significantly with S-LDH-1.[34] In nude mice with transplants of human TGCT type 2, the product of the TGCT mass and the RNA expression of *LDHB* in tumor lesions correlated markedly with S-LDH-1.[35]

Patients with TGCT type 2 and a raised S-LDH have a high proportion of LDH-1.[33–37] The TCGA dataset in our present study showed that EC expressed RNA of *LDHB* higher than the RNA of *CCND2* and *NANOG*. The previous meta-analysis of the 24 candidate genes for TGCT type 2 in the three datasets considered *CCND2* and *NANOG* to be driver genes for TGCT type 2.[16] Hence, due to the

relatively high RNA expression, our study indicates that also *LDHB* is a driver gene for TGCT type 2.

The high RNA expression of *LDHB* is the genetic background for patients with TGCT type 2 and a raised S-LDH have a characteristic high proportion of S-LDH-1.[36,37] Both a systematic review and a critical narrative review document that S-LDH-1 is a relevant serum tumor marker for patients with both SE and EC.[5,38] Adding S-LDH-1 to the TNM classification increased the prognostic prediction for patients with TGCT type 2.[39] *LDHB* has major biologic effects. Knock-down of *LDHB* in lung adenocarcinoma cells reduced growth of the cells.[40] Knock-down of *LDHB* also reduced cell proliferation of maxillary sinus squamous cancer cells.[41] *LDHB* also has important roles for other malignancies.[42,43]

The dramatic effects of expression of *LDHB* reflect that LDH has a central role for anaerobic glycolysis. Anaerobic glycolysis metabolizes intracellular glucose to pyruvate and LDH metabolizes the resulting pyruvate to lactate.[44] Thus, LDH helps malignant cells in the initial steps of glycolysis to produce adenosine 5-triphosphate (ATP) without need of oxygen. Hence, a high LDH activity in malignant cells gives the malignant cells a survival advantage in a hypoxic environment. The Warburg effect[45] explains why more patients with TGCT type 2 and a raised S-LDH die of TGCT than patients with a normal S-LDH.[32,33,37,38] Our study shows that TGCT type 2 expresses RNA of *LDHB* higher than RNA of *LDHA* and *LDHC*. RNA expression of *LDHA, LDHB,* and *LDHC* is regulated by epigenetic mechanisms.[46] In a retinoblastoma cell line, a complete methylation of the promoter for *LDHA* blocked expression of *LDHA*[47] and demethylation with 5-aza-2-deoxycytidine restored RNA expression of *LDHA,* and thereby production of LDHA in the cell line.

Epigenetic mechanisms downregulate *LDHC* in most normal tissues. Methylation of the promoter of *LDHC* inhibits RNA expression of *LDHC*. Transcription factor Sp1 (Sp1) and cAMP responsive element inducing protein 1 (CREB1) regulated gene expression of *LDHC*.[48] Neurofibromin 1 (NF-1) blocked RNA expression of *LDHC*.[49] Correspondingly, adult men have LDH-X in normal testicular germ cells, but S-LDH-X is not detectable in blood.[50] Our study shows that TGCT type 2 downregulates *LDHC*. The downregulation of LDHC explains why patients with TGCT type 2 and raised S-LDH have no detectable S-LDH-X.[37,51]

The 12p has a crucial role for the development of TGCT type 2.[30] SE and NSCGT have an increased copy number of 12p, often as an isochromosome i(12p).[30,34] The analysis of the RNA expression of the 24 genes in the previous meta-analysis and in our present study of the three datasets elucidates that the crucial role of 12p in TGCT type 2 is due to specific genes on 12p.[16] *CCND2, NANOG*, and *LDHB* have gene loci on 12p: 12p13.32, 12p13.31, and 12p12.1, respectively; and *LDHB* is localized close to a candidate amplicon of 12p.[52] Our study shows that TGCT type 2 had higher RNA expression of *LDHB* than that of *CCND2* and *NANOG*. Likewise, previous publications of genes on 12p in TGCT type 2 showed high RNA expression of *LDHB*.[53,54] Hence, the RNA expression of *LDHB* is not solely regulated by the copy number of 12p in the tumors. One of the previous publications also showed that YST highly expressed RNA of *AFP*.[54]

In 2019, a systematic review claimed that there is no evidence for the use of S-LDH in combination with S-AFP and S-hCG in any type of TGCT.[55] However, multivariate analyses of the serum tumor markers in a study of patients with intermediate risk showed that both S-AFP and S-LDH were significant predictors of progression free survival and of overall survival.[11] Zondag and Klein[36] previously found that SE had a unique S-LDH isoenzyme pattern with a predominant S-LDH-1. Correspondingly, future analyses of the TCGA project may investigate whether SE and EC have higher RNA expression of *LDHB* than all other cancer types. In addition, our findings motivate future studies of cell lines of TGCT type 2 where *LDHB* is blocked in the malignant germ cells. Our findings also argue for further clinical studies of S-LDH and S-LDH-1 as serum tumor markers of patients with TGCT type 2. Our study has limitations. It is not a systematic review. Recent guidelines do not include S-LDH-1 as a serum tumor marker of TGCT type 2.[7] No study has analyzed whether posttranslational modifications of LDHB subunits enhance the catalytic activity of LDH observed in other studies.[56]

CONCLUSIONS

TGCT type 2 has a markedly high RNA expression of *LDHB*, the genetic background for LDH-1. Differences in RNA expressions of *AFP, CGB5,* and *LDHB* in the histologic types contribute to discordant levels of the serum tumor markers in patients with TGCT type 2. Our *LDHB* findings support the recommendation for patients with TGCT type 2 with a raised S-LDH that measurements of S-LDH-1 help to determine whether the raised LDH activity is related to the tumors.[36,37]

AUTHOR CONTRIBUTIONS

FEvE made the concept for the study, made statistical analyses, and wrote the first draft of the manuscript. JPA and SMT separately performed reanalyses of the first microarray dataset and contributed actively to the revisions of the manuscript. JPA examined gene expression in data files. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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