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Hepatocellular carcinoma in a gene therapy research subject with ornithine transcarbamylase deficiency

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Abstract

A 66 year old woman who is a manifesting heterozygote for ornithine transcarbamylase deficiency (OTCD) presented with hepatocellular carcinoma (HCC). Fourteen years prior to this presentation she participated in a phase I gene therapy study which used an adenoviral vector, thought to be non-oncogenic, to deliver a normal *OTC* gene to hepatocytes [1]. A recent review of data collected through a national longitudinal study of individuals with urea cycle defects [2, 3] suggests that early urea cycle disorders (UCDs) are associated with hepatocellular damage and liver dysfunction in many cases. This may predispose an affected individual to a substantially increased risk of developing HCC, as has been observed in certain other inborn errors of metabolism. We speculate that the underlying urea cycle defect may be the cause of HCC in this individual.

Keywords

urea cycle disorders (UCDs); ornithine transcarbamylase deficiency (OTCD); hepatocellular carcinoma (HCC); gene therapy; adenovirus

Case Report

The patient is a 66 year old woman with partial OTCD. She was found to be a symptomatic OTC-deficient heterozygote following the diagnosis of OTCD in her son, who presented with hyperammonemia at 6 months of age. He has been managed effectively with dietary protein restriction as well as alternative pathway therapy and is now 29 years old. An

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Disclosure

J.M.W. is a consultant to ReGenX Holdings, and is a founder of, holds equity in, and receives a grant from affiliates of ReGenX Holdings; in addition, he is an inventor on patents licensed to various biopharmaceutical companies, including affiliates of ReGenX Holdings.

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apparently deleterious mutation (p.L77P, c231G>T) was found in the OTC gene of the proband. The patient received a protein load of 1 g/kg at the time of her son's diagnosis in order to determine her carrier status for this X-linked disorder; her ammonia increased from 27 to 92 $\mu\text{mol/L}$ (normal range $<35 \mu\text{mol/L}$) with a concomitant increase in urinary orotic acid from 2 to 63 $\mu\text{g/mg}$ creatinine (normal range $<2 \mu\text{g/mg}$). She had 2 episodes suggestive of clinically significant hyperammonemia, the first at age 25 years when she was on the Stillman diet (high protein) and became extremely lethargic. The second episode occurred at age 37 years when, during the 7th month of the pregnancy with her son, she developed persistent lethargy and vomiting. As these episodes occurred before the diagnosis of her son (her only child), ammonia levels were not obtained. She reported protein self-restriction but has not required other therapeutic interventions, including alternate pathway therapy.

The patient was subject 002 in a gene therapy trial and was the first to receive the adenovirus (Ad) vector in April 1997 at age 52 years (subject 001 temporarily left the study before receiving the gene therapy) [1]. At the time of gene transfer, she had no known history of liver dysfunction and her only medication was estrogen replacement therapy. She does not drink alcohol and has not been exposed to aflatoxin. She had no medical problems but had struggled with obesity for many years and weighed 93 kg at the time of the gene therapy study. As the first subject to receive vector, she was infused with the lowest dose, 2×10^9 particles/kg, of the H5.001CB.hOTC adenoviral vector. There were no abnormalities noted in AST or other laboratory parameters following gene transfer. A liver biopsy taken 7 days after gene transfer identified no acute liver abnormalities. Mild chronic abnormalities were noted and were consistent with those previously reported in OTCD heterozygotes [1]. She remained asymptomatic and was discharged from the General Clinical Research Center one week after vector transfer. At the time of gene transfer, the neutralizing antibody titer to adenovirus serotype 5 was 1:320 and the lymphocyte proliferation index to adenovirus capsids was 12.1 indicating prior exposure to an adenovirus very similar to serotype 5. Follow-up evaluation at 18 months, according to protocol, did not reveal any complications or abnormalities.

In the years since her participation in the gene therapy trial, the patient has developed a number of medical conditions that have strong associations with obesity, including hypothyroidism, type II diabetes, hypertension, congestive heart failure, and coronary artery disease requiring a coronary artery bypass graft at age 62 years. She presented to her physician at age 66 years with fatigue and nonspecific bowel symptoms. An abdominal ultrasound showed abnormalities in the liver, and an MRI of the abdomen was performed. This showed extensive hepatic masses. With enhancement, metastasis seemed less likely, and a primary liver cancer was suspected. Serum alpha fetoprotein (AFP) was elevated at 3,644 ng/mL (normal range $<10 \text{ ng/mL}$). Hepatitis B and C serologies were negative as was anti-nuclear antibody (ANA). A CT-guided biopsy of one of the lesions identified hepatocellular carcinoma. The pathologist noted that the background non-neoplastic liver parenchyma showed portal fibrous extension and a mixed inflammatory cell infiltrate, representing either mass effect or fibrosis in the background liver. Both of these findings have been reported to be associated with OTCD.

Risk of adenoviral vector-mediated oncogenesis

Viral vectors have been evaluated in humans extensively over the past 20 years. In addition to potential acute adverse reactions to viral vector exposure, recipients of viral vectors are considered to be at risk for potential delayed adverse events. The risk of delayed adverse events following exposure to viral vectors is in part dependent on the propensity of the vector to integrate into the host genome, extra-chromosomal persistence of the vector in host cells, prolonged transgene expression, and altered host gene expression. Vectors based on

viruses that integrate into chromosomal DNA, such as murine retroviruses and lentiviruses, were considered to carry a higher potential for cancer-related, delayed adverse events and consequently FDA has included requirements for long-term monitoring of recipients of retroviral-mediated gene therapies since 1993 (Letter to Sponsors of an IND Using a Retroviral Vector, September 20, 1993). Gamma retroviral vectors carrying the IL-2 receptor gamma gene have been implicated in a number of hematopoietic malignancies when delivered to bone marrow by *ex vivo* gene therapy strategies [4, 5].

While wild-type adenovirus serotype 5 has been demonstrated to transform cells *in vitro*, this ability is dependent on the E1a gene, which is deleted in the replication defective Ad5 vector used in our clinical trial [6]. As a result, E1a-deleted adenoviral vectors are not considered high risk for cancer-related adverse events. Moreover, vectors based on adenovirus serotype 5 are considered by FDA to present a low risk for gene therapy-related delayed adverse events since the vectors do not integrate into the genome, do not persist in host cells, and do not stably express their transgenes. Therefore, gene therapy clinical trials involving Ad5 vectors do not generally require long-term follow-up [7]. Very recently, we discovered that a broad range of adenoviruses, including the type used to develop the vector in the OTCD clinical trial, extensively colonize the gastrointestinal tracts of primates including macaque, great apes and humans, apparently without causing disease [8].

Liver disease and hepatocellular carcinoma in OTCD and other urea cycle disorders

At the time of the gene therapy study in the late 1990s, the prevailing wisdom was that extensive hepatocellular damage, liver dysfunction, and liver failure were not associated with proximal UCDs (i.e., carbamyl phosphate synthetase deficiency and OTCD). While this belief persists, multiple case reports in the last 20 years have documented acute liver dysfunction as a clinical presentation of OTCD [9–16]. In recent years, as part of our NIH-funded Rare Diseases Clinical Research Center longitudinal study of UCDs, we have found additional evidence supporting this association. This includes a retrospective chart review of patients treated in our longitudinal study site at Children's Hospital Colorado, in which over half of symptomatic individuals with OTCD (14 of 22) had liver dysfunction or failure [Gallagher et al., unpublished results]. The liver abnormalities found in our patient's hepatocytes that are unaffected by HCC are consistent with findings in other patients with OTCD and are suggestive of the longstanding, chronic liver damage.

Prior to the identification of the current patient, we had raised the possibility that UCDs could be an independent risk factor for primary liver cancer. Two lines of evidence prompted us to consider the association. First, patients with citrullinemia type 2, a disorder of ureagenesis, typically present with mild liver dysfunction, liver fibrosis and fatty infiltration. In the medical literature, three case reports have associated citrullinemia type 2 with HCC [17–19]. In a recent retrospective study, mutations in the gene for citrullinemia type 2, *SLC25A13*, were identified in 12 % of cases of non-viral HCC [20]. Intriguingly, there have been observations indicating a lack of expression of OTC in HCC cell lines with dependence on exogenous arginine for growth [21–23]. Second, HCC appears to be a prototypic primary liver cancer, an apparent result of the increased tumorigenesis in a large number of inborn errors of metabolism causing chronic liver inflammation and fibrosis [20]. We summarized our observations in a recent review paper [24], where we described several potential mechanisms responsible for increased tumorigenesis, including accumulation of toxic metabolites (e.g., tyrosinemia type 1), metabolite shifting (e.g., glycogen storage diseases) and nucleotide imbalance (e.g., deoxyguanosine kinase deficiency).

Based on these mechanisms, UCDs, in particular OTCD and argininosuccinic aciduria, could increase the risk of liver cancer for two reasons:

1. Chronic liver damage by toxic metabolites, as evidenced by elevated plasma amino transferases (ALT and AST).
2. Excess of carbamoyl phosphate being either toxic itself (due to high energy phosphate) or alternatively diverted into the pyrimidine synthesis pathway, resulting in dysregulation of the nucleotide pool – a phenomenon observed in mitochondrial depletion syndromes and glycogen storage diseases – both of which have been linked to primary liver cancers, as noted above.

To test this hypothesis, prior to and then subsequent to the identification of the current patient, we searched the UCD longitudinal study database, contacted the National Urea Cycle Disorders Foundation (the patient advocacy organization), and screened and posted inquiries through the metabolic listserv Metab-L. We have identified four patients with UCDs, 3 in the US and 1 in the UK, who are currently being treated for hepatocellular carcinoma. In addition to the patient in this report they include: a 31 year old female with OTCD, a 67 year old female with OTCD, and a 4 year old with argininosuccinic aciduria (personal communications).

Based on our longitudinal study of over 450 patients in 11 sites in the US [2] combined with the number of prescriptions filled for sodium phenylbutyrate (Buphenyl, Ucylyd Pharma, personal communication), we believe there are approximately 2,000 patients with UCDs currently being treated in the US and the UK. This would provide an estimated incidence of HCC of about 200 per 100,000, while the occurrence of HCC in the general population is 1–3 per 100,000 [25]. This actually may be an underestimate of HCC in UCD as due to the life-threatening nature of UCDs, many patients, especially those who have the most severe neonatal onset disease, undergo liver transplantation in infancy. Therefore, we may be managing UCD patients' liver cancer risk in the most severely affected patients by explanting the affected organ.

We hypothesize that those patients with less severe forms of UCD (e.g., OTCD heterozygous and late-onset forms of all UCDs) who typically are not considered for liver transplantation could be at increased risk for developing a primary liver cancer later in life. Whether we need to incorporate screening for hepatocellular carcinoma (i.e., abdominal ultrasound and serum AFP) into routine surveillance of these patients remains to be determined by future studies.

Conclusion

It is unlikely that the adenoviral vector administered to this patient 14 years ago contributed to her HCC. Humans are extensively colonized with latent adenovirus genomes due to natural infections without any association with cancer [8]. The vector used in this trial does not integrate or persist and extensive experience with replication defective adenovirus vectors in animals and humans has not revealed an increased risk of any malignancy. Furthermore, it is doubtful that there was any appreciable hepatocyte transduction in this patient. She was the first recipient of the vector receiving the lowest dose (2×10^9 p/kg) which was 250-fold lower than the dose of vector which showed minimal transduction in monkeys [26]. In addition, her pre-existing neutralizing antibody titer to adenovirus serotype 5 was 1:320 which has been shown to substantially reduce hepatocyte transduction [1, 26].

Our recent evaluation of this patient provided an opportunity to revisit the potential contribution of UCD in the development of HCC. These studies suggest that there may be a previously underappreciated association of UCDs with liver dysfunction which may be a

precursor for an apparent increased risk of HCC in these disorders. One could consider several changes in the management of patients with UCD if the association with HCC is substantiated in further studies such as screening procedures and the avoidance of activities such as alcohol intake that could exacerbate the risk. An increased risk of HCC in UCD should also be considered in the development of new therapies. This was evaluated in the mouse model of OTCD in the context of gene therapy vectors based on adeno-associated viruses which persist but do not efficiently integrate; there was no increased incidence of malignancy above background with vectors expressing the normal OTC gene [27]. This may not be the case with vectors or other biopharmaceutical agents which carry a known independent risk of malignancy which in the case of gene therapy would include vectors based on retroviruses.

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Highlights

- Urea cycle disorders (UCDs) are attractive candidates for gene therapy especially those treatable by targeting liver.
- We describe a research subject with a deficiency of ornithine transcarbamoylase who participated in a gene therapy trial 14 years ago who recently developed hepatocellular carcinoma (HCC). The cause of HCC in this person is likely due to the underlying disease and not gene therapy.
- Prior to the development of cancer in this person we speculated that UCDs lead to chronic liver inflammation and may pre-dispose to HCC.
- A world-wide survey of patients with UCDs suggested a 100-fold increase in the risk of developing HCC as compared to normal subjects.
- Further studies are warranted to determine if patients with UCDs undergo routine screening for HCC.