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World's First Myoblast Treatment of Human Cancer Found Safe and Efficacious

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Abstract

Evolution of placental mammals over the past 160 million years witnesses the relative sparing of muscles from cancer attacks. In 1) nude mice with human gastrointestinal or lung tumors, and 2) human subjects with liver, lung or gastrointestinal tumors, intra-tumor implantation of allogeneic human myoblasts induced cancer apoptosis, inhibiting metastasis and tumor growth. We postulate four mechanisms of cancer apoptosis: a) myoblasts releasing tumor necrosis factor-α (TNF-α); b) deprivation of nutrients and oxygen; c) local inflammatory and immunologic attacks; and d) prevention from metastasis. These basic and clinical studies demonstrated preliminary safety and efficacy of intra-tumor myoblast implantation in the development of prevention and treatment for cancer, now the number one disease killer of mankind.

Keywords

Human Cancer Treatment, Tumor Shrinkage, Cancer Apoptosis, Metastasis Inhibition, Cancer Clinical Trial, TNF-α, Myoblasts, Myotubes, Nude Mice, Cell Therapy

1. Introduction

Myoblast transplantation is the world's first human gene therapy and somatic cell therapy that corrected the primary gene defect of Duchenne muscular dystrophy boys in year 1990 [1]. It has great social and economic value [2] [3], considering its potential application in treating fatal and debilitating hereditary diseases such as muscular dystrophies [4] [5], cardiomyopathies [5] [6] [7] [8] [9], Type-II diabetes [5] [10] [11], and cancer of human beings.

Skeletal myoblasts have a unique ability to fuse. Implanted myoblasts naturally fuse among themselves to form genetically normal myofibers to *replenish* dead fibers [1] [5] [7] [8] [9] [10] [11]. They also fuse with dystrophic [1] [5], diabetic [5] [10] [11] skeletal myofibers, and ischemic cardiomyoctytes [12] [13], inserting their nuclei that carry the normal genome to produce genetic complementation *repair*. It is in this unique ability of natural cell fusion that we discovered four mechanisms of cancer inhibition with myoblasts.

In the 500 million years of vertebrate evolution, especially during the 160 million years of mammalian divergence of placental and marsupial [14], the skeletal muscle, being externally located in the body, has developed specific characteristics for frontline defense against predation including carcinogens. Despite the involvement of most differentiated cell types in the human body, reports of primary or metastatic tumors in cardiac, skeletal and smooth muscles have been rare. This led us to believe that the myogenic cells might have developed certain mechanism(s) to defy carcinogenic insults in the course of evolution. Apart from radiation, chemo and cell therapies of human invention over the past 120 years that are largely inadequate, we now present an out-of-the-box approach, utilizing the natural and unique phenomena of myoblast cell fusion and the release of cancer-killing factors that have developed over 160 million years of placental evolution.

The discovery of a muscle development promotion factor called cachectin or tumor necrosis factor-α (TNF-α) [15] [16] in muscle prompted us to test the effect of human myogenic cells on cancer cells, initially using myoblasts as they are muscle precursors. The myoblasts were obtained from primary culture of satellite cells isolated from muscle biopsies of male volunteer donors devoid of blood-borne pathogens, after obtaining Institutional Review Board (IRB) approval and Donor Inform Consent [1] [17] [18].

In year 1995, Law disclosed the results of co-cultures of normal human myoblasts and malignant melanoma (CRL6322) cells [19]. After 5 days culturing in the myoblast fusion medium, melanoma cells became spherical, apoptotic and detached from the collagen surface, whereas myoblasts either began to fuse or had already fused to become myotubes. At 10 to 19 days in myoblast fusion medium, numerous dead melanoma cells were floating on the culture medium surface. Myoblasts and myotubes were developing vigorously on the collagen. Significantly more myotubes were observed after 19 days in the myoblast fusion medium when myoblasts and melanoma cells were seeded initially at 3:1 concentration ratio. Whereas the myoblasts undergoing cell fusion and the newly-formed myotubes appeared to have induced cancer cell apoptosis, they were not able to have completely extinguished the cancer cells in culture [19].

The switching from culture medium to fusion medium constituted a condition of serum restriction because the fusion medium contained only one-fifth of the serum concentration as in the culture medium. Serum restriction terminated the mitotic cycle of the myoblasts, and initiated the developmental process of natural cell fusion towards myotube formation. Law envisioned that myoblast

fusion was associated with membrane breakage with significant amount of TNF-α and possibly other cancer killing factors being released. Furthermore, myoblast fusion resulted in myotube formation and development, quickly depriving the melanoma cells of oxygen and nutrients within the confined microenvironment of the tumor capsule. These two mechanisms were considered to be responsible for the melanoma cell death in the co-culture studies [19].

TNF-α is an endogenous muscle factor promoting myogenesis through activation of the p38α and Pax7 pathway [20]. TNF-α mRNA basal level in C2C12 myoblasts had been shown up-regulated 273% by serum restriction [21]. TNF-α synthesis and secretion in muscle [22] [23] showed marked increases upon strenuous exercise [23] [24]. By activating the JNK pathway, it triggers cancer apoptosis through a caspase 3 dependent pathway [25].

In 2013, Stolting *et al*. reported that myoblasts restricted prostate cancer growth and metastasis by paracrine TNF-α secretion. An increase up to 25 fold of TNF-α mRNA basal level was demonstrated when myoblasts were co-cultured with tumor cells. Co-culture experiments revealed induction of cell cycle arrest, tumor death by apoptosis and increased myoblast differentiation. This effect was largely blocked by TNF-α inhibition. The same outcome was noted in nude mice, in which co-injected human myoblasts inhibited the tumor growth and lymph node metastasis of all prostate cancer cell lines evaluated [26].

Based on the above information, we initiated a series of animal experimentation leading to a clinical study testing the feasibility, safety and preliminary efficacy of implantation of allogeneic human myoblasts into solid tumors of cancer patients as follows.

2. Materials and Methods

2.1. Manufacture of Human Myoblasts

2.1.1. Muscle Donors

Upon approval of the Institutional Review Board (IRB) of the Cell Therapy Institute and the signing of the Donor Informed Consent, muscle donors were admitted after meeting the Inclusion and Exclusion criteria. They were male volunteers between the ages of 13 and 27. They were certified by a physician as being in good health, having normal levels of aspartateaminotransferase (AST), alanine transaminase (ALT), or lactate dehydrogenase (LD) and tested negative for human immunodeficiency virus (HIV), hepatitis B surface antigen (HBSAg), hepatitis C (HCV), syphilis (RPR), and cytomegalovirus (CMV-IgM). They also received the following tests: Chem 24, CBC, and physical examination with normal results. Donors were excluded if they had any chronic or infectious diseases, or were allergic to the local anesthetic Lidocaine.

2.1.2. Muscle Biopsy

About 2 grams of muscle were removed from the quadriceps muscle using an open biopsy technique under local anesthetic (Lidocaine) in a sterile field of a surgical suite of a hospital. The donor site was sutured and bandaged. No prophylactic antibiotic was used. The donor was discharged after recovery from

the surgical procedure to be followed by his physician if infection occurred.

2.1.3. Preparation of Myoblasts

Biopsy specimen obtained was processed immediately using sterile techniques meeting CFDA approved GMP and ISO 9001 standards. Myoblasts were cultured in growth medium and incubated in 35˚C - 37˚C and 7% CO_2 as previously described [17] [18]. Myoblasts were frozen at different stages so the time allotted for culturing could be coordinated with a scheduled transplant. The amount of cells frozen and the number of samples were documented. One test vial was reserved in liquid nitrogen for each biopsy.

Random samples of the myoblasts were tested for their ability to divide, fuse, and form myotubes [17]. Lot release testing consisted of sterility, endotoxin, mycoplasma, and testing for myoblast identity, purity, potency, viability, and cell count on a pooled sample prior to transplant meeting quality control standards [17]. A retain sample of myoblasts was frozen in liquid nitrogen from each transplant.

2.2. Animal Studies

To determine biologic dosing and pharmacokinetics, nude mice were used to test the effect of human myoblasts on the proliferation and apoptosis of non-small-cell lung cancer (NSCLC) A549 cells, and of human gastrointestinal cancer cells SGC-7901 in subcutaneous solid tumors. In addition, human myoblasts were injected into tumors having Ehrlich ascites cells (BS344 EAC) in KM mice to determine if such intervention might prolong the life-spans of the cancer inflicted mice.

Male BALB/c nude mice averaging 17 ± 1 g were obtained from Beijing Witung Lihua Limited Co. SCXK (Beijing) 2008-0005. Male KM mice averaging 20 ± 2 g were obtained from Hubei Animal Experimentation Center SCXK (Wuhan) 2014-0007. Mice were maintained in compliant with SPF standards. NSCLC and EAC were supplied by the Alfie Inc., Wuhan. Six animal studies were conducted as listed in **Table 1**.

2.2.1. Study 1

Involved 20 nude mice injected subcutaneously on each side of the back with 0.2 mL of A549 NSCLC cells at a concentration of 25 million/ml. After 18 to 20 days when the tumors reached 250 - 300 mm^3 in volume and had developed capillary network of their own, 0.2 ml of saline was injected into the left tumor and 0.2 ml of human myoblasts into the right tumor at a low concentration of 10 million/ml of saline.

The length and width of control and test tumors were measured every week using a caliper. The volume was calculated using the formula: (length × $width^2$)/2. At 3 weeks after myoblast treatment, mice were sacrificed and the tumors dissected out and weighted. Student's t-tests demonstrated significant difference at $P < 0.05$ between the mean volumes (2687.8 ± 713.4 mm^3 vs. 2133.7 ± 638.3 mm^3) and weights (3.12 ± 0.88 g vs. 2.48 ± 0.78 g) of control versus myob-

Table 1. Dosing and pharmacokinetic studies of human myoblasts injected into subcutaneous mature solid tumors of nude mice established with human non-small-cell lung cancer (NSCLC) A549 cells (Study 1) and with human gastrointestinal (GI) cancer cells SGC-7901 (Studies 2 to 5). Study 6 compared the survival periods of myoblast-treated KM mice previously injected with Ehrlich ascites cells versus control.

Studies	Cancer	Cell Line	No. of Mice	Myoblasts/Volume	Days Follow-up
1	NSCLC	A549	20	2×10^6/0.2 ml	21
2	GI	SGC-7901	8	6×10^6/0.15 ml	5
3	GI	SGC-7901	22	2×10^6/0.15 ml 10×10^6/0.15 ml	15 4
4	GI	SGC-7901	5	14×10^6/0.15 ml 6×10^6/0.15 ml	4 5
5	GI	SGC-7901	6	28×10^6/0.15 ml	9
6	EAC	BS344	11	50×10^6/0.2 ml	22
	EAC	BS344	10	0/0.2 ml	17

Table 2. Effects of human myoblasts on the volume and weight of NSCLC A549 tumors in nude mice (n = 20).

Group	Volume (mm^3)				Weight (g) x ± SD (n = 8)	TIF (%)
	d0	d7	d14	d20		
Control	305.5 ± 78.6	1032.8 ± 310.4	1875.3 ± 546.8	2687.8 ± 713.4	3.12 ± 0.88	
Test	325.8 ± 101.4	874.2 ± 276.5	1586.0 ± 488.7	2133.7 ± 638.3*	2.48 ± 0.78*	20.5

TIF, tumor inhibitory factor; *indicates $p < 0.05$ by Student's t-test.

last-treated tumors (Table 2). Study 1 demonstrated that injection of allogeneic human myoblasts into solid tumor of nude mice inhibited mature lung cancer growth, reducing tumor volume and weight by 20.6% and 20.5% respectively, despite the use of low dosage of 2 million human myoblasts at a low concentration of 10 million/ml.

2.2.2. Studies 2 to 5

Consisted of four groups of nude mice aged 4 to 5 week old that had previously received subcutaneous injections of GI SGC-7901 cancer cells on the back and had developed mature tumors of similar sizes on both sides measuring approximately 0.3 × 0.2 × 0.2 cm. These dose-escalation studies were designed to study the pharmacokinetics of myoblasts to determine the safety and efficacy of treating gastrointestinal cancer using different myoblast concentrations and procedures.

For example, Studies 2 and 5 involved single-time injections of 6 and 28 million myoblasts with follow-up periods of 5 and 9 days respectively. Studies 3 and 4 involved two-time injections of 12 and 20 million myoblasts with follow-up periods of 19 and 9 days respectively.

In these studies, comparison was made between myoblasts-injected tumors versus control tumors that received similar volume of carrier solution, in terms of tumor size and cancer cell number as revealed by histology of tumor sections

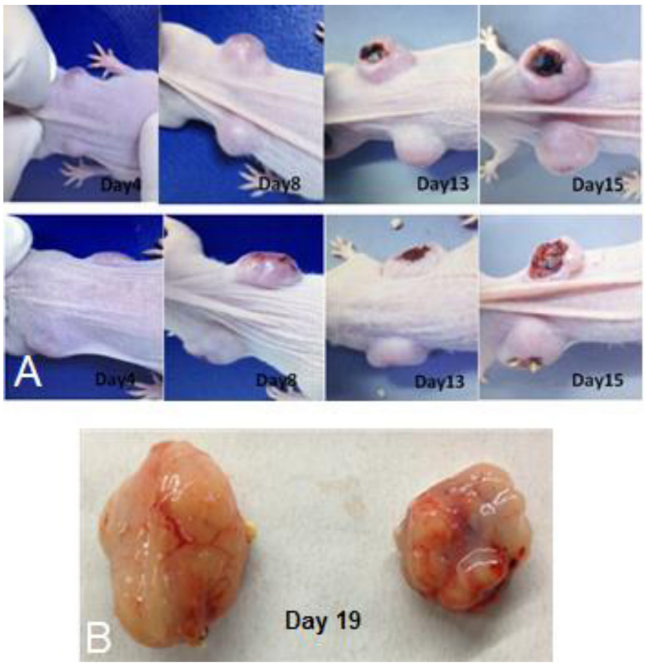

Figure 1. Intra-tumor Myoblast Injection Inhibited Cancer Proliferation and Tumor Growth in Two Representative Lung Cancer-inflicted Nude Mice. (A) On Day 1, injection of 2×10^6 allogeneic human myoblasts into test tumors (top) led to relative increases in tumor sizes until Day 8 as compared to control tumors receiving saline (bottom). This is because of myotube formation within the first week of myoblast transplant [27]. Significant reduction in volumes of test tumors was observed in Day 13 as compared to controls. Whereas test tumors decreased in size from Day 8 to Day 13, control tumors showed significant increases in sizes. Additional implantation of 10×10^6 myoblasts on Day 15 boosted the volumes of the test tumors. (B) Administration of the myoblast regime in Study 3 using a total of 12×10^6 myoblasts reduced the volume of the test tumor (right) to about 50% of the control (left) after 19 days.

using H&E stain.

Figure 1(A) showed two representative mice from Study 3 each had received 2 million myoblasts in 0.15 ml for the initial 15 days followed by a second injection of 10 million myoblasts in 0.15 ml on day 15. The mice were sacrificed on day 19, and representative control and myoblast-treated tumors were shown in Figure 1(B). Myoblast injection had inhibited cancer proliferation and reduced the volume of the tumor to about 50% of the control.

Histologic study demonstrated spindle-shaped myoblasts and myotubes amidst round tumor cells in the myoblast-treated tumors (Figure 2(A), Figure 2(B)). Abundant cancer cell death appeared as empty space or vacuoles in the tumor sections. As usual, sections of control tumors are compact with tumor cells (Figure 2(C)). Newly-formed myotubes, unable to become innervated and vascularized inside the tumor capsule, had perished, leaving empty space and vacuoles also. Myoblasts immuno-stained brownish with human desmin were

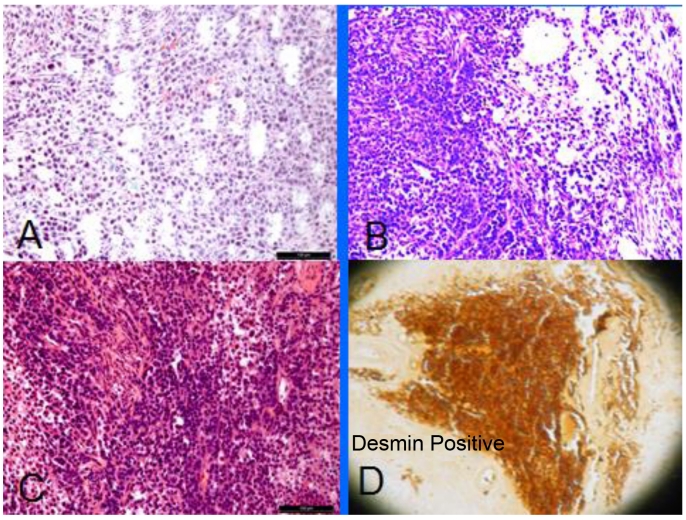

Figure 2. Histologic study of the tumors in **Figure 1(B)** demonstrated ((A), (B)) presence of spindle-shaped myoblasts and myotubes amidst round tumor cells in the myoblast-treated tumor, with deaths of cancer cells and non-vascularized myotubes appearing as empty space or vacuoles in the tumor sections. (C) Section of the control tumor compact with cancer cells. Microscope magnification for (A), (B), and (C) is ×200. H&E stain. (D) Myoblasts stained brownish with human desmin immunocytochemistry were observed wrapping around the round cancer cells in sections of the myoblast-treated tumors but not in sections of control tumor. Microscope magnification for (D) is ×400.

observed wrapping around the round cancer cells in sections of the myoblast-treated tumors (**Figure 2(D)**), but not in sections of control tumors.

Study 2 to 5 established the safe and effective dose range and optimal pharmacokinetics of the allogeneic human myoblasts in treating mature gastrointestinal cancer using different myoblast concentrations and procedures. Doses of 2 to 28 million myoblasts administered at 13.3 million/ml to 186.7 million/ml respectively were found to be safe and effective in reducing tumor volume, weight and cancer cell number. The surprising discovery of myoblasts wrapping around the cancer cells in sections of the myoblast-treated tumors (**Figure 2(D)**) suggested a fourth mechanism of cancer cell apoptosis, namely that the myoblasts prevented the cancer cells from metastasis, and continued to exert detrimental effects on them.

2.2.3. Study 6

Involved 21 KM mice injected intraperitoneal with 20 million Ehrlich ascites cellseach and randomized into test (11 mice) and control (10 mice) groups. One day later, control mice each received intraperitoneal injection of 0.2 ml of saline, whereas test mice each received 0.2 ml containing 50 million human myoblasts. The mean survival period after cancer implantation for the control mice was 15.4 ± 1.5 days, significantly less than that for the myoblast-injected mice of 18.6 ± 3.2 days at $P < 0.005$ by Student's t-test. Study 6 demonstrated that 50 million allogeneic human myoblasts administered at a high concentration of 250 mil-

lion/ml could extend the lifespan of mice inflicted with immature Ehrlich ascites cells by 20.8%.

3. Postulated Mechanisms of Cancer Cell Apoptosis

Four mechanisms were considered to be responsible for inhibition of cancer cell proliferation, tumor volume reduction and cancer cell apoptosis:

1) The tumor necrosis factor-α (TNF-α) released following cell membrane breakage in the processes of myoblast mitosis and cell fusion killed cancer cells (Figure 3(A));

2) Dividing myoblasts and newly developed myotubes competed successfully and had taken away most if not all of the nutrients and oxygen inside the tumor from the cancer cells (Figure 3(B));

3) Injection trauma of allogeneic myoblasts mounted local inflammatory and immunologic attacks on both myoblasts and cancer cells (Figure 3(C)); and

4) Myoblasts wrapped around cancer cells, preventing them from metastasis, and continued to exert detrimental effects on them (Figure 2(D)).

4. Basis to Initiate Clinical Trial

Results of co-culture and animal studies supported the hypothesis that mitotic or fusing human myoblasts and newly formed myotubes were potent biologics to

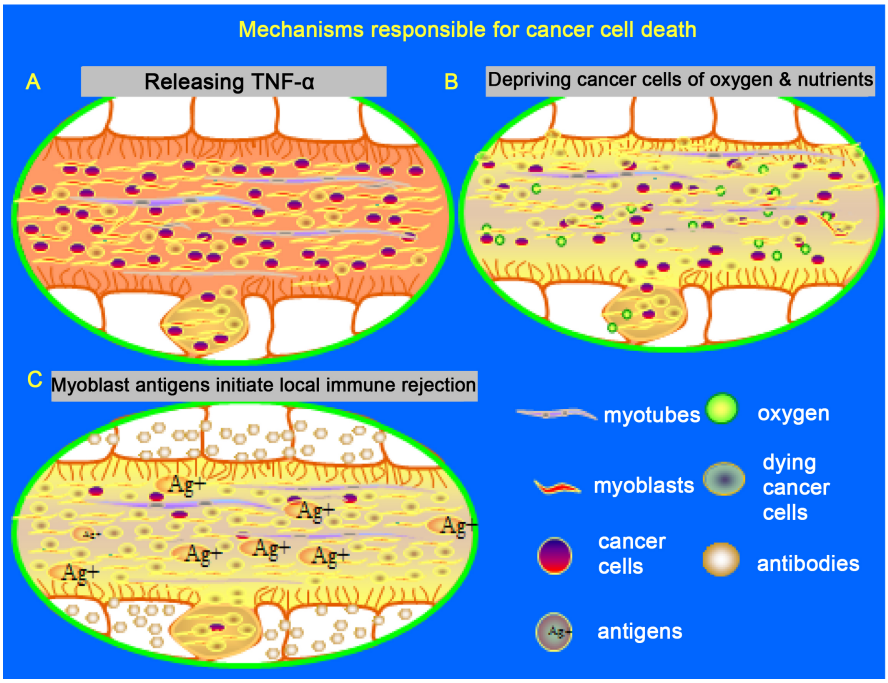

Figure 3. Initially, three mechanisms were considered to be responsible for inhibition of cancer cell proliferation and cancer cell apoptosis: (A) tumor necrosis factor-α (TNF-α) released following cell membrane breakage during myoblast mitosis and cell fusion killed cancer cells; (B) dividing myoblasts and newly developed myotubes competed successfully and had taken away most if not all of the nutrients and oxygen inside the tumor from the cancer cells; (C) injection trauma of allogeneic myoblasts mounted local inflammatory and immunologic attacks on both myoblasts and cancer cells.

inhibit cancer cell proliferation, killing cancer cells and inhibiting tumor growth. Considering that our terminal cancer subjects had no immediate effective alternative, and the demonstrated safety of myoblast treatment of patients suffering muscular dystrophy, cardiomyopathy, and Type II diabetes, benefit versus risk ratio would favor proceeding onto clinical studies with cancer patients.

5. Clinical Trial

In China, cell transplantation is considered as a medical treatment technology and has been regulated not by the Chinese Food and Drug Administration (CFDA) but by the National Ministry of Health, now called the National Health and Family Planning Commission. As of July 2, 2015 the Commission abolished the necessity to gain approval at the national level for somatic cell transplantation to initiate clinical trials, except for stem cells. Such human studies, however, have to be approved by a Grade 3A hospital that would take on the responsibility of patient safety and register such studies with the Health and Family Planning Commission at the provincial level [28]. The CFDA still has to approve the plant of cell manufacture.

The use of allogeneic human myoblasts as a biologic in clinical studies was approved by the Institutional Review Board (IRB) of the Third Affiliated Hospital of Xinxiang Medical University in Henan, China.

Three volunteer cancer patients, aged between 55 and 80, were admitted after meeting the Inclusion and Exclusion criteria and signing of Patient Informed Consent. They were certified by a physician as being in good health, having normal levels of AST, ALT, or LD, and tested negative for human immunodeficiency virus (HIV), hepatitis B surface antigen (HBSAg), hepatitis C (HCV), syphilis (RPR), and cytomegalovirus (CMV-IgM). They also received the following tests: Chem 24, CBC, and physical examination with normal results. Subjects were excluded if they had any infectious diseases.

Being the world's first, this clinical trial proceeded with great caution, examining the safety and efficacy of precision implantation of allogeneic human myoblasts into solid tumors of three patients having lung, liver and gastrointestinal cancers respectively.

5.1. Case 1

Yang XX, female, aged 62, had history of lung cancer metastasized into the brain and the left adrenal gland. Her brain metastasis was treated previously with radiation therapy and chemotherapy for 3 weeks without remission.

The subject underwent allogeneic human myoblast implantation into the adrenal metastatic small cell carcinoma of the lung on September 10, 2015. MRI showed the tumor from 49.50 mm in its maximum length measured at one month before (**Figure 4(A)**) developing to 52.50 mm at the time of myoblast implantation. Implantation was guided with a General Electric (GE) Vivid E9 Color Doppler Ultrasound after piercing with a needle through the abdominal cavity. About 1 billion allogeneic myoblasts at a concentration of 100 million/ml

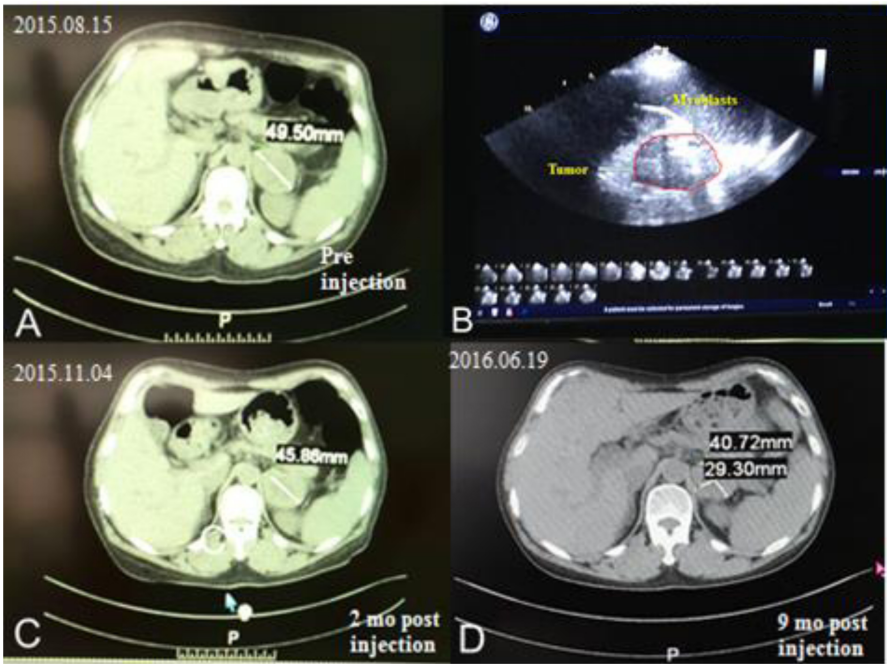

Figure 4. (A) MRI showed the adrenal metastatic small cell carcinoma of the lung measured 49.50 mm in its maximum length at one month before implantation. (B) About 1 billion allogeneic myoblasts at a concentration of 100 million per ml of patient's own serum were injected into one side (light) of an oblong solid tumor measuring over 52 mm in length. The outline of the adrenal metastatic small cell carcinoma of the lung is traced in red. The other side (dark) of the tumor was not injected and served as a control. (C) At 2 months after myoblast implantation, the tumor size decreased, measuring 45.86 mm in length. A second implantation was administered at 2.5 months after the first. (D) The decrease in tumor size continued until 9 months after the first implantation, with the tumor length being measured at 40.72 mm.

of the patient's own serum [29] were injected into one side of the oblong solid tumor. The other side of the tumor was not injected and served as a control (**Figure 4(B)**).

Some adverse reactions were observed, treated and remised in 10 days. These included temporary reduction in blood pressure down to 82/50 mmHg, coughing, phlegm sputum and headache.

The abdomen was examined with MRI (Siemens, Magnetum-ESSENZA) before and after myoblast implantation, comparing tumor size and density through signals obtained from test and control areas. Examining methods included Axi: IN-PHASE, OPP-PHASE, TSE T2WI/FS, DWI and Cor: TRUFI, T2WI.

At 2 months after myoblast implantation, the tumor size decreased, measuring 45.86 mm in length (**Figure 4(C)**). A second implantation of 1.4 billion allogeneic myoblasts was administered at approximately 120 million/ml on November 26, 2016 with the hope to further interrupt cancer proliferation and to induce cancer apoptosis. The patient suffered no adverse reaction. The tumor length decreased to 40.72 mm at 9 months after the first implantation (**Figure 4(D)**).

Pathology of the adrenal tumor biopsies at 2 months postoperatively con-

firmed the diagnosis of adrenal metastatic small cell carcinoma of the lung, with TIF-1 (+), Vimentin (−), CK (pan) (−), CK7 (−), CK19 (−), SyN (+), CgA (+), Ki-67 (+, 40%). Histologic examination demonstrated that the non-injected portion of the carcinoma was densely packed with cancer cells (Figure 5(A)). Large scale of cancer cell death was apparent in the myoblast-injected portion of the tumor (Figure 5(B)).

5.2. Case 2

Wang, XX, male, aged 67, previously diagnosed with cardiac malfunction and primary liver cancer having multiple tumors, underwent allogeneic human myoblast implantation on September 10, 2015. About 700 million myoblasts at a concentration of 100 million/ml of patient's own serum was injected into a solid tumor measuring 35.2 mm × 25.2 mm (Figure 6(A)). Implantation was guided with a General Electric (GE) Vivid E9 Color Doppler Ultrasound after piercing with a needle through the upper abdominal cavity and the right anterior wing of the liver where the treated tumor was located. Another tumor in the right posterior wing of the liver, measuring 28.9 mm × 25.4 mm × 26.7 mm, was left untouched to serve as control (Figure 6(E)).

The upper abdomen was examined with MRI (Siemens, Magnetum-ESSENZA) at 1 month after myoblast implantation, comparing tumor size and density

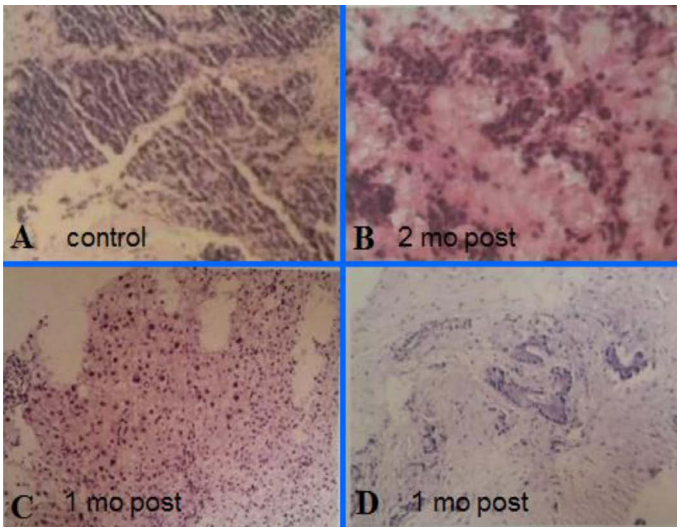

Figure 5. At 2 months after myoblast implantation, (A) histologic section of the non-injected portion of the adrenal metastatic small cell carcinoma of the lung was densely packed with cancer cells. (B) Large scale of lung cancer cell death was apparent in the myoblast-injected portion of the tumor. (C) Histologic section of T1 liver tumor demonstrated nodular connective tissue distributed among sclerotic liver tissue 1 month after myoblast implantation. No cancerous tissue was observed. (D) Cancer cell scarcity with the presence of myotubes were observed at 1 month after myoblast injection in a histological section of a metastatic adenocarcinoma of the abdominal wall from a 63-year-old man with gastrointestinal cancer. Microscope magnification ×400; H&E stain.

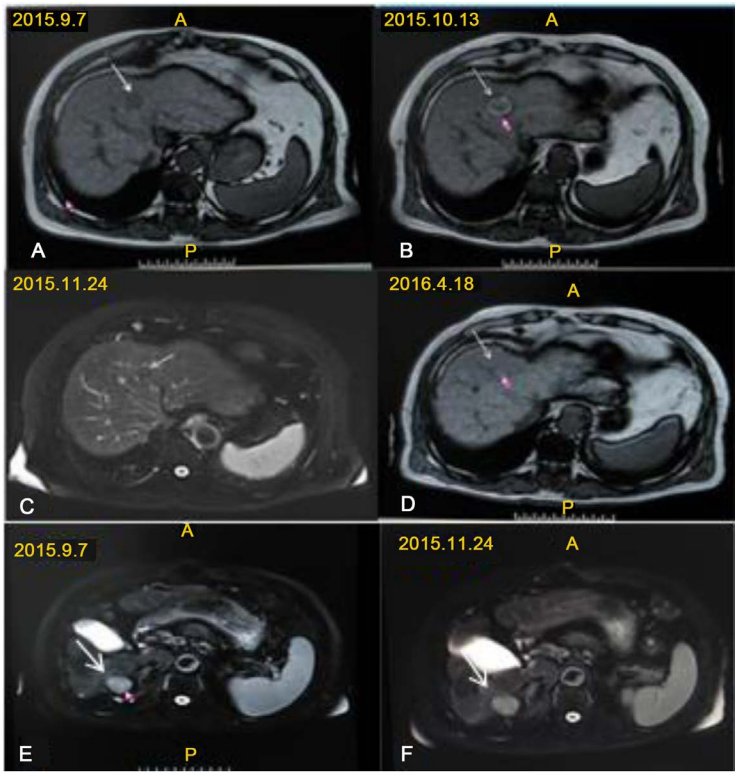

Figure 6. MRI of liver cancer showing the size and density of the upper tumor (arrow) before myoblast implantation (A), and at (B) 1 month, (C) 2.5 months, and (D) 7 months after implantation. Whereas (E) the non-injected control lower tumor (arrow) in the same liver increased in size in 2 months (F), the myoblast-injected T1 showed a significant decrease in size and density with time, indicating that the myoblasts and myotubes interrupted cancer proliferation and induced cancer apoptosis.

through signals obtained from test and control areas. Examining methods included Axi: IN-PHASE, OPP-PHASE, TSE T2WI/FS, DWI and Cor: TRUFI, T2WI.

MRI demonstrated that the myoblast-injected upper tumor significantly decreased in size and density with time (**Figures 6(A)-(D)**), whereas the non-injected control lower tumor in the same liver increased in size (**Figure 6(E)**, **Figure 6(F)**). This indicated that the implanted myoblasts and developing myotubes interrupted cancer proliferation and induced cancer apoptosis.

At 1 month after myoblast implantation, the myoblast-injected tumor was punctured and two needle biopsies were obtained measuring 1.5 cm and 2.0 cm in length respectively. Pathology demonstrated nodular connective tissue being distributed among sclerotic liver tissue. No cancerous tissue was observed (**Figure 5(C)**), indicating that the implanted myoblasts and developing myotubes had interrupted cancer proliferation and induced cancer apoptosis.

5.3. Case 3

Shang XX, male, aged 63, had previous been diagnosed with gastric cardia high/middle differentiated ulcer type adenocarcinoma that became metastasized to

the abdominal wall and the lymph node posterior to the diaphragm. Immunopathology demonstrated CEA (+), Villin (+), CK19 (+), CK7 (+/−), CK20 (−), Ki-67 (+, 30%), CKpan (+). Surgical removal and chemotherapy did not result in remission of the cancer.

On December 23, 2015, the subject underwent allogeneic human myoblast implantation into the metastasized tumor on the abdominal wall measuring 41.5 × 16.9 mm, and into the metastasized tumor in the lymph node measuring 29.0 × 23.0 mm. About 400 million myoblasts at a concentration of 100 million/ml of patient's own serum were injected into each tumor without any adverse reaction.

At 1 month after myoblast implantation, there was a slight reduction in tumor sizes measuring 29.0 × 23.0 mm and 19.0 × 12.0 mm respectively. Histology of the abdominal tumor showed cancer cell scarcity with the presence of myotubes (**Figure 5(D)**).

As of March 11, 2017 when this manuscript was submitted, all the myoblast-treated patients were alive and well.

6. Discussion

Cumulated results from co-culture studies, animal experimentation and clinical trial provided confirmatory evidence to indicate that myoblasts and developing myotubes, either singly or in combination, were potent biologics that inhibited tumor growth and induced cancer cell apoptosis.

Direct injection of allogeneic (or even autologous) human myoblasts at 100 million per milliliter of host serum into the solid tumor without immunosuppressant was preferred, though myoblast concentration might vary from 75 million to 250 million per milliliter.

Exposing the allogeneic myoblasts to 100% host serum primed the myoblasts for proliferation. Implantation of this mixture into the tumor constituted serum restriction, a condition that terminated mitosis and induced cell fusion to occur.

Myoblasts' unique characteristic shared only with cardiac and smooth muscle cells was natural cell fusion through which myoblasts at the end of their mitotic cycle underwent cell membrane breakage, releasing large but naturalquantity of cancer-killing TNF-α and possibly other TNFs into the microenvironment. The second phase of cell fusion was accomplished by massive sarcolemma formation, enclosing two hundred to five hundred myoblast nuclei into one myotube. Competition for nutrients and oxygen against cancer cells within the tightly encapsulated tumor was fierce resulting in death of cancer and myogenic cells. Each of the myotube had to be vascularized and innervated to survive, failing which the myotubes would disappear, leaving vacuoles and empty spaces within shrunken tumors. Furthermore, allogeneic myoblast implantation triggered inflammation and local immune response, killing myoblasts and cancer cells indiscriminately. Cancer cells also became non-metastatic as being "wrapped" with myoblasts immuno-stained brownish with desmin.

7. Conclusion

This is the first report to have documented plausible mechanisms and the use of

precision implantation of intra-tumor myoblast implantation to treat solid tumors in cancer patients. The safety and efficacy that it demonstrated, though preliminary, lead the way to developing a potential new treatment for cancer. Lacking graft-versus-host damage, myoblasts and developing myotubes are safe biologics. Obviously the benefit versus risks ratio will favor well designed clinical trials to be conducted at their earliest, including randomized, double-blind studies.

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