Comprehensive Clinical Plasma Medicine

Cold Physical Plasma for Medical Application
submission. As a matter of course our profound gratitude to all authors for their substantial contributions and professional cooperation!

And we are especially grateful for your personal getting involved with these reflections before starting to wander through the chapters. That looks like a keen and rewarding interest in clinical plasma medicine. We wish you success!

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Cold Atmospheric Plasma (CAP) Combined with Chemo-Radiation and Cytoreductive Surgery: The First Clinical Experience for Stage IV Metastatic Colon Cancer


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9.1 Introduction

Colorectal cancer (CRC) is the third most common cancer in the world and the second leading cause of cancer death in the United States. In 2012 an estimated 103,170 new cases of colon cancer and approximately 40,290 rectal cases were newly diagnosed with 51,690 related deaths from these combined cancers [1]. There is evidence of peritoneal carcinomatosis (PC) in 8–10% of these patients at the time of diagnosis and 25% during the progression of their disease [2–5].

PC is associated with a poor prognosis. Patients are considered to have a terminal condition with a 6–10 month median survival time [2–7]. The standard treatment for advanced stage CRC and PC is systemic chemotherapy which is considered palliative with minimal improvement in patient survival. Advanced chemotherapeutic regimens such as FOLFOX have been reported to improve survival to a median of 15.7 months [8, 9].

Cytoreductive surgery (CRS) combined with hyperthermic intraoperative peritoneal chemotherapy (HIPEC) has evolved over the past 20 years as a new approach for the treatment of PC. CRS is described as removal of gross tumor follow by HIPEC treatment. Despite limited evidence to support CRS and HIPEC, there are some reports that this new approach has reported beneficial results [10]. Although there are promising results, CRS and HIPEC is associated with a significant morbidity, mortality, increase operating time, prolonged ICU care which results in an increase cost in patient care. This new multimodality approach is limited to several factors; age, extra abdominal disease (liver or lung metastasis), and peritoneal cancer index (PCI) which is the most common prognostic indicator and relies on the spread of the disease based on a scoring systems and the capability of complete removal of the gross tumor. PCI score calculates the spread of tumor in 13 areas of the abdomen in combination with tumor size. It ranges from 0 to 39 points.
An elevated score indicates significant increase tumor load [11]. Elias et al. [12] reported a 4 year survival rate of 44% if the PCI score is <6, score between 7 and 12 (22%) and >19 (7%) respectively. CRS and HIPEC is not recommended if the PCI score is >20. Controversy still exists whether CRS and HIPEC is considered “experimental”.

Plasma medicine has qualified as a new scientific field after intense research effort in low-temperature or cold atmospheric plasma applications [13–15]. It is known that cold atmospheric plasmas (CAP) produce various chemically reactive species including reactive oxygen species (ROS) and reactive nitrogen species (RNS). CAP is a cocktail containing ROS and RNS in combination with transient electric fields, UV and charged species.

CAP has already been proven to be effective in wound healing, skin diseases, hospital hygiene, sterilization, antifungal treatments, dental care, and cosmetics targeted cell/tissue removal [16–18]. One of the most recent applications of CAP is in cancer therapy [19–21]. Multiple studies have convincingly demonstrated that the CAP treatment leads to selective eradication of cancer cells in vitro and reduction of tumor size in vivo. While most studies were done in vitro, some work was done in vivo [1–3, 7–9]. Recently, clinical cases of CAP application in cancer therapy were presented at the 2nd International Workshop on Plasma for Cancer Therapy in Nagoya (Japan) [22, 23] and one of these studies involving 12 patients afflicted with advanced squamous cell carcinoma of the head and neck has been documented in a recent paper [24].

The authors report a novel treatment approach for peritoneal carcinomatosis secondary to colon cancer using Cold Atmospheric Plasma combined with chemotherapy, radiation and cytoreductive surgery.

### 9.2 Clinical Patients Descriptions

A 56 year-old female previously underwent a laparoscopic right hemi-colectomy on April 18th 2011 at a local hospital in Lanham, MD. Pathology revealed adenocarcinoma, moderately differentiated with focal mucinous areas arising in villous adenoma at cecum proximal ascending colon and positive for one out of seven regional lymph nodes (T3N1M0). She was originally treated with adjuvant chemotherapy FOLFOX after primary resection but developed an allergic reaction to oxaliplatin toward the end of treatment. She received two courses of FOLFIRI. In December 2012, CT scan revealed a solitary liver lesion and extensive tumors throughout the abdomen consistent with peritoneal carcinomatosis. She has a mutated K-ras (codon 12). Her CEA was 3014.5, CA19-9 2682 on 11.27.13.

Patient was referred for consultation on November 2013. Her CT scan showed widespread peritoneal carcinomatosis Stage IVb (T3N1M1b). PET scan performed on November 2013 showed hepatic metastasis, numerous omental and abdominal wall masses, hyper-metabolic masses in the right lower quadrant next to the colon, mass at the ileocolonic anastomotic site intraluminal respectively, and increase activity in the pelvis posterior to uterus, cul de sac consistent with metastatic disease. A multi-disciplinary approach was started to cytoreduce the gastrointestinal tumors.
Patient received 14 cycles of FOLFIRI with the last dose on 9.22.14. Patient was subsequently treated using IMRT to all sites of disease based on 3D Conformal Computerized Contours combined with Xeloda. Nine gantry angles were used for the abdominal areas and eight gantry angles were used for the pelvic gutters. Summary of radiation treatment entailed abdominal regions dose 4500 cGY, 25 fractions from March 6th 2014 to April 24th 2014, Pelvic Gutters 4500 cGY 25 fractions from March 6th 2014 to April 23rd 2014. CT scan of chest, abdomen and pelvis on June 2014 showed good response to chemotherapy with a decrease in size of peritoneal metastatic implants with the exception of lesions on the surface of the spleen and nodule in the pelvis.

9.2.1 Methods

The Institutional Review Boards at Baton Rouge General Hospital (Bluebonnet campus), Baton Rouge reviewed and approved the study protocol. Approval was obtained from the Food and Drug Administration (FDA) for Compassionate and Emergency Use prior to surgical treatment as well as informed surgical consent. Human tissue was handled according to the tenets of the Declaration of Helsinki. SS-601 Electrosurgical generator integrated with Canady Plasma™ Coagulator and Canady Hybrid Plasma™ Scalpel (US Medical Innovations, LLC (USMI) Takoma Park, MD) was used for gross dissection of tumor and afterwards the Canady Helios™ Cold Plasma Ablator (USMI Takoma Park, MD) was used to treat the margins at the tumor site. March 2015 the patient underwent exploratory laparotomy, liver segmentectomy, cholecystectomy, right partial diaphragm resection with reconstruction using allograft patch, en bloc resection of distal small bowel, transverse, left colon, sigmoid colon, distal pancreas with spleen and omentum, small bowel resection, resection of tumors from the mesentery and abdominal wall and supracervical total abdominal hysterectomy with bilateral salpingoophorectomy. A R0 resection was completed. Time of procedure and estimated blood loss were 7.5 h and 800cc respectively. Specimens sent to pathology were positive for metastatic adenocarcinoma from the liver, peritoneal implants, small bowel tumor with implants in the mesentery, bilateral tumor involvement adjacent to the ovaries, en bloc resection of the transverse, left and sigmoid colon, prior anastomosis to the small bowel, spleen with the tail of the pancreas, multiple tumor deposits of the mesentery, mesocolon, peripancreatic, perisplenic, adipose tissue and the small bowel prior anastomotic staple line. The patient’s peritoneal cancer index (PCI) was >23 intra-operatively.

9.2.2 Hospital Course

After surgery the patient was transferred to the ICU and subsequently transferred to the floor. Patient was taken back to the operating room on 3.22.15 for anastomotic leak at the ileoproctostomy site. Take down of the rectal anastomosis and Brook
ileostomy was performed. Patient returned to the OR on 3.24.15 and 3.27.14 for abdominal washing of abdomen and closure of the fascia. Postoperatively the patient developed an enterocutaneous fistula which was managed by TPN and abdominal wound vac. Patient was discharged to home on 5.15.15. On June 25th, 2015 postoperative CT scan of the abdomen and pelvis revealed no evidence of tumor in the abdomen. Patient and family decided hospice care August 2015.

### 9.3 Treatment of the Resected Surgical Margins Using Cold Atmospheric Plasma (CAP)

In the course of surgery, CAP treatment of the surgical margins (diaphragm, abdominal wall, mesentery, left colic gutter, mesenteric area, area of the splenic bed) was performed using the Canady Helios Cold Plasma™. Images of treatment of surgical margins by CAP are shown in Fig. 9.1c, d. Figure 9.1c show partial resection of the diaphragm using the Canady Hybrid Plasma™ scalpel and cold plasma jet treatment of surgical margins after resection are shown (Fig. 9.1d).

![Fig. 9.1](image)

(a) OR set up. (b) Cold Plasma Generator (USMI). (c, d) Partial resection and Cold plasma treatment of surgical margins, ex vivo. (e) Treatment of liver. (f) Treatment of diaphragm
In addition to treatment of surgical margins we performed cold plasma treatment of the ex vivo sample of liver (shown in Fig. 9.1e) and diaphragm (shown in Fig. 9.1f). Treated and untreated samples were imaged and analyzed using various assays (see Sect. 9.5).

### 9.4 Body Temperature Measurements During Surgery

In this section we present body temperature measurements during surgery using USMI Helios cold plasma device. Schematics of experimental set up is shown in Fig. 9.2.

#### 9.4.1 Experiment Setup and Procedure

A thermal camera (FLIR A35) with a 19 mm lens and a 60 Hz frame-rate was used to collect the plasma object and patient body’s thermal data. The FLIR A35 camera was mounted approximately 3 ft above the patient to observe the treatment area. To get a better viewing angle and to ensure that the camera is less intrusive to the surgeon’s procedure, a less than 20° view angle was applied to the camera. The FLIR A35 thermal camera’s area of view at approximately 3 ft is 2.7 ft by 2 ft area, which is larger

![Fig. 9.2](image)
than the 1 foot by 1 foot of the patient’s procedure area. In total, 5 h of thermal video was captured by the camera and stored onto the hard drive of the control PC. During the procedure, the patient was treated with the Canady Hybrid Plasma™ and the Canady Helios Cold Plasma™ scalpels. The treatments consisted of “spraying” the margins of the cancerous area with the cold plasma jet created at the distal end of the Helios Cold Plasma Scalpel. The settings for the Canady Helios Cold Plasma™ Ablator’s settings were 1.6 W and helium flow rate 5 L/min for a duration of 2 min per treatment area.

The data captured by the FLIR camera was later processed using FLIR tools+[25]. To compare the cold plasma intraoperative thermal performance and patient’s tissue reaction to the cold plasma scalpel, the patient’s pre-treatment area tissue temperature and post-treatment tissue area temperature were measured. Tissue area temperature was calculated by taking the average of the temperature data in the treatment area by using the built in functions of FLIR tools+, Fig. 9.3 demonstrate a side by side comparison of tissue temperature pre-treatment and post-treatment.

Along with intraoperative cold plasma treatments, the thermal camera recorded several cold plasma ex vivo treatments as shown in Fig. 9.1. The tumor cells and a small amount of normal cells were removed from the patient for a comparison study; tumor and control samples were treated with the cold plasma scalpel with the same settings as the intraoperative treatments. The pre-treatment and post-treatment thermal images for ex vivo were processed identically to the intraoperative treatments as shown in Fig. 9.4.

During the data processing, FLIR tools+ built in functions were selected to measure the treatment area’s minimum, maximum, and average temperatures. Based on the background reference material [25], a thermal emissivity of 0.95 was selected, so as to best represent the actual temperature of live body tissues for the spectral range of the camera (7.5–13 μm). During the procedure, the patients End Tidal CO₂ and O₂ level were recorded via the ventilator and the pulse oximeter respectively.

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**Fig. 9.3** Thermal stills of the cold plasma intraoperative treatment. The left figure is the pre-treatment tissue temperature; the right figure is post-treatment tissue temperature.
9.4.2 Experiment Results

Thermal measurements from the in vivo treatments are shown in Fig. 9.5. Significant temperature change occurs in the area of treatment for each case. A temperature drop of 10–20° across the surface of the tissue occurs in all six treatments. Temperature measurements of the surrounding tissue and tissues pre-treatment are displayed as well and are consistent with the core temperature measured with the hospital’s equipment.

Fig. 9.4 Thermal stills of the cold plasma ex vivo treatment. The left figure is the pre-treatment tissue temperature; the right figure is post-treatment tissue temperature

Fig. 9.5 Graph of intraoperative temperature, showing the temperature before and after treatment as well as the temperature measured by the OR equipment
9.5 Expansion In Vitro, Characterization and Immunohistological Analysis of Human Primary Colon Cancer Epithelial Cells Isolated After Surgery

In this Section we describe characterization of the human primary colon cancer cells. Identification of colon stem cell markers CD44 and TRAIL receptor 1 were performed (Figs. 9.6 and 9.7)

![Ex Vivo Sample Temperature: Post and Pre Treatment](image)

**Fig. 9.6** Ex vivo cold plasma treatment results, showing the temperature measured before and after the treatments. The thermal measurements for the ex vivo are shown in figure. There is a slight temperature decrease between pre-treatment and intraoperative treatment, but not as large as in the case of the patient.

![Intraoperative O2 and End Tidal CO2 during Cold Plasma Treatment](image)

**Fig. 9.7** O₂ and End Tidal CO₂ results for the patient during the treatments. The patient intraoperative O₂ and End Tidal CO₂ measurements were unaffected by the treatment and were well within the normal range throughout the treatments as shown in figure.
9.5.1 Materials and Methods

9.5.1.1 Human Tissue Preparation and Sample Collection

Human tissue was handled according to the tenets of the Declaration of Helsinki. On the basis of our studies in vitro using primary LT-97-3 colon cancer stem cells (Generous gift of Dr. Brigitte Marian, Univ. of Vienna Medical Center, Austria; J Pathol. 2007 Oct;213(2):152–60), HCT-116 ATCC derived Colon cancer cells and normal colon epithelial cells (unpublished data) we chose to use the LT-97-3 medium for developing primary cultures. Medium used to culture the human LT-97 includes the following components, 4 parts Ham F12, 1 part L15, 2% FCS, insulin, 20 nm Triiodotyronin, Trasnferrin-20 μL for 500 mL, 1 μg/mL hydrocortisone- 20 μL, 30 ng/mL EGF- 15 μL, Penicillin 10,000 μg/mL (5 mL) / Streptomycin (5 mL)/gentamycin (2.5 mL) (Sigma Aldrich). The samples were collected in 20 mL sample collection vials containing the above medium at 4 °C and brought back to the lab within 10 h from the sample collection site and processed immediately.

Colon tumor explants was minced to 1 mm size and was processed to isolate epithelial cells using enzymatic digestion with 1 mg/mL collagenase type IV for 10 min at 37 °C and some of the 1 mm samples were expanded in vitro as explant cultures. The human colon epithelial cultures were expanded in a BSL2 classified laboratory (Jerome Canady Research Institute for Advanced Biological and Technological Sciences, Takoma Park, MD) for maintaining cell lines for biomedical, translational and regenerative biology applications. Five freshly isolated human colon cancer samples were procured within 10 h of patient’s surgery in sterile 20 mL borosilicate sample collection vials containing the above mentioned medium composition. 3–5 cm² or larger size of the colon tumor and normal tissue samples excised from the patient were used in the current study: (1) liver tissue with colon cancer treated with CAP (2) liver tissue with colon cancer treated without CAP (3) Subphrenic Diaphragm with colon cancer treated with CAP (4) Subphrenic Diaphragm with colon cancer treated without CAP and (5) normal diaphragm with and without CAP. Tissues were treated with penicillin streptomycin in PBS and minced and processed as described previously [26]. Normal diaphragm tissues were processed for cryosectioning and H&E staining. These two methods of cell culture namely, explant cultures and isolated cells cultures were used in the current study to generate primary using the patient’s biopsy samples. Only two explants developed into epithelial cultures from tissues isolated with enzymatic treatments. The cells were serially diluted (into six 35 mm well plates) with the hope that the stem cells would develop and proliferate into colonies. All cultures were terminated for the following tests for histology, confocal microscopy to detect various proteins/antigens. Images were acquired periodically to assess the morphology of the cells.
9.5.1.2 Immunofluorescence Analysis for Identification of Colon Stem Cells and Colon Cancer Markers in Tissues Excised Using Cold Plasma Scalpel

Some of the tissues procured from the patient were immediately cryosectioned using Leica cryostat. 5–6 µm sections at −20 °C were stained with H&E and double immunofluorescence for localization of colon stem cell marker human CD44 FITC (Bio Legend), anti TRAIL receptor1 (Santa Cruz) and second antibody anti-alexaflour 594 or 488(molecular probes), respectively was used along with nuclear counterstaining with DAPI ( Vectashield, Molecular Probes). Appropriate isotype controls (Life Technologies) were maintained. Zeiss confocal images were acquired to analyze the cold plasma excised tissue for remnant colon cancer markers.

9.5.1.3 Confocal Imaging

Zeiss 1um tick Z-stack images were acquired and 3D–reconstruction of the images (Jerome Canady Research Institute for Advanced Biological and Technological Sciences, Takoma, Park, MD) were analyzed for surface expression of TRAIL-R1 and CD44 or Ki67 in the cryosections (n = 3) and in cultured colon cancer cells after 30 days in culture. The entire dish was assessed and images were captured for the remnant cells and the % of positive cells was calculated. 15 images per dish were acquired to record the % total number of cells remaining and % of cells positive for the above markers.

9.5.1.4 Statistical Analysis

The following test was carried out for n = 3 samples.

Nonparametric Tests: Independent Samples. NP tests/independent Test-Mann Whitney Wald Wolfowitz Kruskal Wallis test compared pair wise; median(test value = sample = compare = pairwise) Hodges Lehmann/missing Scope = analysis usermissing = exclude/criteria with alpha = 0.05 Cilevel = 95.

9.5.2 Results

9.5.2.1 Metastatic Liver With and Without CAP Treatments

Figure 9.8 shows cross sections images along the thickness of the sample excised from the patient. Note the intactness of the tissue sections in a and b showing no damage at the site of CAP treatments (arrow). Arrowhead indicates the metastatic colon cancer area in the liver. These tissues were used to isolate the colon cancer cells to be cultured and expanded in vitro for further analysis.
Fig. 9.8 (a, b) H&E stained showing (a) Metastatic liver treated with cold plasma (b) without Cold Plasma

9.5.2.2 Metastatic Tumor from Sub Phrenic Diaphragm With and Without CAP Treatments

Figure 9.9 shows images, which are cross sections along the entire thickness of the sample excised from the patient. Note the intactness of the tissue sections in a and b showing no damage at the site of CAP treatments (arrow). Arrowhead indicates the metastatic colon cancer area in the sub phrenic diaphragm. These tissues were used to isolate the colon cancer cells to be cultured and expanded in vitro for further analysis.
9.5.2.3 Normal Diaphragm With and Without CAP Treatments, to Demonstrate That CAP Does Not Cause Injury to the Healthy Tissues

Images in Fig. 9.10 are cross sections along the thickness of the sample excised from the patient. Note the intactness of the tissue sections in a and b showing no
damage at the site of CAP treatments (arrow). Arrowhead shows muscle fibers of the diaphragm separated by loose connective tissue.

9.5.2.4 Confocal Double-Immunofluorescence Images of Human Liver Showing Localization of Colon Stem Cells (CD44 Positive Red) and TRAIL-Receptor 1 (Green)

Figures a, b are samples without CAP treatments and figures c, d are with CAP treatments. Note the bright TRAIL R-1 staining in (c and d) in presence of CAP and the absence of bright TRAIL-R1 in (a and b).

Freshly procured human tumor samples from liver treated with and without CAP were oriented and embedded in the cryostat. 6–7μm thick sections and fixed in ice-cold methanol for 15 min and double-immunostained for TRAIL-R1 and CD44. In the presence of CAP treatments, the TRAIL-R1 expression increases. Moreover, the double positive cells expressing CD44 and TRAIL-R1, typical of a stem cell was observed in all the tumor samples as shown in Fig. 9.11a–d. It was found that the localization and expression of TRAIL-R1 in the CAP treated CD44 positive cell was greater in both number and expression (c, d). These results suggest that CAP triggering of TRAIL-R1 in colon cancer stem cells may play a role in apoptosis. Therefore, we isolated and expanded the cells from these CAP treated and untreated tumor samples to further characterize the cellular profile.

9.5.2.5 In Vitro Expansion of Human Colon Cancer Cells from Liver Samples Treated with CAP

In order to test for characterization of cellular profile generated from tissue explants with and without CAP, two different in vitro culture methods were employed (materials and methods). It was interesting to note that explant cultures of liver did not show any outgrowth of cells, while cells isolated using enzymatic treatments, yield a varied population of cells. Figure 9.12 demonstrate that the CAP treated tissue yielded a population of epithelial cells, which over a period of time showed morphology of mostly differentiated cells in the terminal phase of apoptosis. Most of the cells were floating in culture demonstrating an apoptotic phenotype.

9.5.2.6 In Vitro Expansion of Human Colon Cancer Cells from Liver Samples Without CAP Treatment.

The explant cultures showed no outgrowth, while the cells isolated from liver samples showed small colonies and proliferating colonies as shown in Fig. 9.13 (arrowhead a, b). This colony was tracked and the colony size increased over time (a, b). After more than 3-weeks of culture these cells were still proliferating (arrowhead). These cultures were terminated to assess the molecular characteristics using various markers.

9.5.2.7 In Vitro Expansion of Human Colon Cancer Cells from Subphrenic Diaphragm Samples Treated with CAP

The cellular profile and phenotype from CAP treated sub phrenic diaphragm (Fig. 9.14) showed epithelial cells going through apoptosis. However, the explant
Immunofluorescence for CD44(red) and TRAIL-R1(green) showing Colon Cancer stem cells (arrow) in metastatic Liver.

**Fig. 9.11** Confocal Immunofluorescence images of human Liver demonstrate localization of colon stem cells (CD44 positive red) and TRAIL-Receptor 1 (green) (arrow-a to d). DAPI was used for nuclear counterstaining.

 Cultures showed fibroblast-like cells, which may not be of colon cancer origin and may be from the remnant diaphragm tissue. The isolated cell cultures showed floating dead cells at the end of 27th day.

**9.5.2.8 In Vitro Expansion of Human Colon Cancer Cells from Sub Phrenic Diaphragm Samples Without CAP Treatment**

Sub phrenic diaphragm without CAP treatment (Fig. 9.15) showed colon epithelial stem cell phenotype. The small colony enlarged over a period of time showing colon stem cell phenotype (b, d, f). However, the explant cultures showed fibroblast-like cells, which may not be of colon cancer origin and may be from the remnant diaphragm tissue.
Fig. 9.12  In vitro expansion of Human colon Cancer cells from liver samples treated with CAP. Representative images of explant (a) and isolated cell cultures (b–d) of colon cells from liver showing cell death and many floating dead cells (arrow) and differentiated cells (arrowhead) in the presence of CAP after 17–27 days in culture (b–d). Note the absence of outgrowth in the liver explant cultures.

Fig. 9.13  Representative images of isolated cell cultures of colon cells from liver showing healthy proliferating colony of cells (arrowhead) without CAP after 17 (a) to 24 days (b–d) in culture.
**Fig. 9.14** In vitro expansion of Human Colon Cancer cells from Sub phrenic Diaphragm samples treated with CAP. Explant (a, c, e) and isolated cell cultures (b, d, f) of colon cells from Sub phrenic Diaphragm showing cell death and many floating dead cells (arrowhead) in the presence of CAP after 17–27 days in culture. Arrow shows fibroblast-like cells from the tissue explants.

### 9.5.2.9 Characterization of Colon Cancer Cells Expanded In Vitro Isolated from Liver

Isolated cell cultures of colon cells from liver showing disintegrating nuclei of smaller size in (arrowhead in a, b) Note these cells are negative for Ki67 and TRAIL-r1, suggesting that these cells are in last phase of apoptosis. (b) Showing healthy proliferating colony of cells (arrow; green Ki67 positive and red-TRAIL-R1 positive) without CAP after 30 days in culture.

Most of the cells display disintegrated nuclei and a few differentiated large cells in the presence of CAP treatment as shown in Fig. 9.16. The smaller size of the nuclei and absence of any cytoplasmic material was suggestive of a dead cell phenotype after CAP treatment. Large number of proliferating Ki67 positive cells were observed in cultures without CAP treatment and such cells were more in number when compared to the CAP treated. These results suggested that the profile of cells after CAP treatment was significantly different from the healthy proliferating tumor cells, generated from samples without CAP treatment. Therefore in order to further
Fig. 9.15  In vitro expansion of Human Colon Cancer cells from Sub phrenic Diaphragm samples without CAP treatment. Cellular profile of the sub phrenic diaphragm showing proliferating colony of cells (arrow) without CAP after 17–27 days in culture (b, d, f). Note the large colony, a property of a tumor stem cell. Explant cultures show fibroblast-like phenotype.

Fig. 9.16  Characterization of colon cancer cells expanded in vitro isolated from liver nuclear counterstaining with DAPI (blue)
characterize these cells for their proliferation we measured Ki67 along with TRAIL-R1 and the % total of positive and double positive cells in CAP treated and untreated cells was calculated.

9.5.2.10 Characterization of Cellular Profile of Human Colon Cancer Cells Expanded In Vitro

Our analysis suggests that all the cells went through cell death and apoptosis in the CAP treated case. Recall that this proliferative population of cells was absent in the CAP untreated samples. About 40% cells were proliferating (Ki67 positive) and 24% cells expressed TRAIL-R1 in CAP untreated, while CAP treatment lead to cell death and apoptosis as shown in Table 9.1. None of the CAP treated cells expressed TRAIL-R1 and were not proliferating, suggesting that these cells were no longer viable and their apoptotic mechanism was initiated by CAP treatment. These results suggest that CAP has an effect in inducing colon stem cell death by triggering TRAIL Receptor -1 expression.

9.6 Concluding Remarks

The authors report the first use of cold atmospheric plasma in a clinical setting for the treatment of metastatic stage IV colon cancer and demonstrate the safety and efficacy of cold atmospheric plasma.

The human colon cancer in the patient samples expressed colon stem cells in liver and was positive for CD44 and TRAIL-R1 expression. TRAIL-R1 expression increases in the CAP treated liver tissues, suggesting that the death receptor molecule may be involved in inducing apoptosis. Isolation of colon epithelial cells from these liver and subphrenic diaphragm explants after CAP treatment, induced cell death within 3 weeks of culture. CAP untreated tissues yields a population of cells that are healthy and colonies increase in size, typical of tumor stem cells. These colonies proliferated even after 4 weeks in culture. In addition explant cultures after treatments with CAP and without treatments yielded fibroblast-like cell phenotype only in the subphrenic diaphragm samples and not from liver explants. These results suggest that these may be normal healthy cells and not the colon cancer epithelial cells and may require further investigation. It has to be noted that none of the CAP treated cells after 3-weeks of culture expressed TRAIL-R1. Moreover, the nuclei of

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<th>Table 9.1</th>
<th>Characterization of cellular profile of human colon cancer cells expanded in vitro</th>
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<td></td>
<td>%Double positive cells</td>
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<td>Colon tumor cells without CAP*</td>
<td>24</td>
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<td>Colon tumor cells with CAP</td>
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The entire culture dish was analyzed to calculate proportion of total number of cells. Significant (*p≤0.05) difference in the profile was cells with CAP treatment and without treatment was observed. Note the absence of TRAIL-R1 and Ki67 positivity in CAP treated samples.
remnant cells were all disintegrated and were significantly different from the population of CAP untreated proliferating Ki67 positive cells.

Overall the results suggest that CAP has an effect on colon epithelial cells and colon stem cells and induces tumor cell death and the use of CAP had no adverse event to the patient.

References


