CASE REPORT – OPEN ACCESS

International Journal of Surgery Case Reports 26 (2016) 42–46

Contents lists available at ScienceDirect

International Journal of Surgery Case Reports

journal homepage: www.casereports.com

A new complementary procedure for patients affected by head and neck cancer: Chemo-predictive assay

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ARTICLE INFO

Article history:
Received 9 May 2016
Received in revised form 13 June 2016
Accepted 12 July 2016
Available online 15 July 2016

Keywords:
Drug response assay
Chemosensitivity assay
ChemoID assay
Cancer stem cells
Oral cancer

ABSTRACT

INTRODUCTION: Administration of ineffective anticancer therapy is associated with unnecessary toxicity and development of resistant clones. Cancer stem cells (CSCs) resist chemotherapy, thereby causing relapse of the disease. Thus, development of a test that identifies the most effective chemotherapy management offers great promise for individualized anticancer treatments. We have developed an ex vivo chemotherapy drug response assay (ChemoID®), which measures the sensitivity of CSCs as well as the bulk of tumor cells to a variety of chemotherapy agents to assist an oncologist in making treatment decisions.
METHODS: Three patients affected by oral cancer were referred.
RESULTS: Biopsy showed a well-differentiated squamous cell carcinoma (G1) in case 1, a G2 adenocarcinoma in case 2 and a G3 squamous cell carcinoma in case 3. In all of the three cases, after clinical inspection and suspicion of a diagnosis of cancer, a double biopsy was performed. One specimen was sent to the ChemoID laboratory for chemosensitivity assay and the other for histological analysis. Chemotherapy dose response curves were generated, and grouped in 3 categories: 1. No response (less than 30% cell kill), Intermediate (30–60% cell kill), and 3. Sensitive (60% cell kill or above).
CONCLUSIONS: This procedure may be useful in helping physicians choose an effective chemotherapy regimen for head and neck cancer patients and lower treatment costs by eliminating ineffective chemotherapies and unnecessary toxicity particularly in elderly patients.

1. Introduction

Because of difficulties in treatment of head and neck malignant tumors, investigation and development of novel strategies and

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http://dx.doi.org/10.1016/j.ijscr.2016.07.013

integrated therapies are required to find more effective treatments for these malignant tumors [1,2].

Patients with the same stage and grade of cancer may vary considerably in their clinical response to chemotherapy [3]. Ineffective anticancer therapy can result in unnecessary toxicity and the development of resistant clones. The surviving cancer cells are often more resistant to therapy [4]. Many attempts have been made over the years to develop an ex-vivo anti-cancer test that could help in discerning the best treatment options for each individual patient while minimizing toxicity [4,5].

Animal xenograft models have shown that only a subset of cancer cells within each tumor is capable of initiating tumor growth. This biological behavior, first observed in AML, has been extended to a multitude of solid tumors, including breast, glioblastoma, colorectal, ovarian, head & neck, and pancreatic cancers [6–9].

This pool of cancer cells is operationally defined as the "Cancer Stem Cell" (CSC) subset. According to the "cancer stem cell" theory, tumors are a complex, growing population of abnormal cells originating from a minority of CSCs [6–9]. These cells maintain stem-like characteristics in that they proliferate very slowly and have an inherent capacity to self-renew and differentiate into phenotypically heterogeneous, aberrant progeny [9–11].

Unlike the bulk of tumor cells, CSCs resist chemotherapy and radiation therapy and are responsible for tumor relapse and metastasis [4,6,7,10–14]. Targeting CSCs in addition to the bulk of other cancer cells within a tumor is a new paradigm in cancer treatment [15].

Our recent studies show that selectively enriched CSCs from primary cancer cell cultures can be used in a chemosensitivity assay (ChemoID®) [4,11].

ChemoID® is a proprietary drug response assay, which measures an individual's malignant tumor response to arrange a standard-of-care anticancer drugs therapy under consideration by a physician [4,12]. ChemoID® uses one of the most advanced technologies to identify chemotherapies that can eliminate the CSCs that are the root of the cancer behavior and relapse [4,12]. ChemoID® is a test that quantifies an individual cancer patient's tumor response to various chemotherapeutic drugs against cancer stem cells and bulk of tumor cells providing both sensitivity and resistance information that can help the Oncologists to tailor the best chemotherapy cocktail directed against the patient's cancer cells. This equates to "individualized" chemotherapy based on the specific patient's own tumor characteristics to give patients an edge against cancer [4,5,12–15]. ChemoID® can be used for selecting the first, second or third line chemotherapies, whether the aim of chemotherapy is cure or palliation.

The aim of this study was to demonstrate the feasibility and efficacy of the ChemoID® chemo predictive assay, which is performed in a CLIA accredited laboratory in the United States for patients affected by extensive lesions of the oral and maxillofacial area who are located and treated in Europe, thus opening new prospective treatment of extensive cancer lesions where a technology such as ChemoID® is not available yet, by just performing two specimen biopsies immediately at the beginning of the treatment.

2. Material and methods

2.1. Chemotherapy agents

Bevacizumab (Avastin), Cisplatin, Methotrexate, 5-Fluorouracil, Docetaxel, Bleomycin, Epirubicin, Gemcitabine, Paclitaxel, Carboplatin, Cetuximab were acquired as clinical grade chemotherapy agents.

2.2. Methods for collecting biopsy specimen from oral cavity and shipping modalities

We have developed a specimen collection procedure that involves a thorough disinfection of the oral cavity before the biopsy, followed by a subsequent disinfection of the collected specimen with a Betadine solution, followed by a wash of the specimen with a sterile saline solution before placing in the transportation medium. Fresh biopsy specimens were shipped using courier services in a specially designed temperature controlled styrofoam container packaging. Biopsy specimens were received at the ChemoID laboratory in the United States within 18–36 h from Europe.

Samples were transported in sterile test tubes containing RPMI-1640 culture media with 2% Penicillin/Streptomycin. The professionally designed cardboard packaging followed international standards for impact durability and safety for the shipment of biological materials.

For international shipments, a lead sheet of 2.5 mm thickness was wrapped around the biopsy collection vial to shield from radiation exposure of airport x-ray scanners. The radiation shielding lead sheet ensured biopsy viability for recovering optimum live cells for the assay.

Another innovative aspect of the collection process that we developed is the implementation of a methodical aseptic procedure at the time of biopsy collection, particularly for oral cancers. Biopsy specimens taken from the oral cavity are usually very high in bacterial and fungal content even after several chlorhexidine rinses. To minimize contamination problems, we developed a decontamination procedure that consists of thorough disinfection of the oral cavity prior to the biopsy, followed by a subsequent disinfection of the specimen with a Betadine solution. This was followed by a meticulous wash of the specimen with a sterile saline solution to remove the Betadine disinfectant.

2.3. Patients

The study was conducted in accordance with the ethical principles provided by the Declaration of Helsinki and the principles of good clinical practice, under the IRB protocol number 695141. Case 1 was a 55 years old woman, heavy smoker, obese, without any history for other pathology, who consulted our the Department of Medicine and Surgery, Unit of Maxillofacial Surgery, University of Salerno, Salerno, Italy for a neoplastic ulcer of the left buccal mucosa of about 6 cm in diameter, infiltrating the muscles of the cheek. Clinical examination revealed pain in the involved area.

The patient didn't suffer from human immunodeficiency virus (HIV-1) infection. She did not complain of bleeding, and no other significant complaints were present in her clinical history. On Computerized Tomography (CT) exam there was clear presence of the tumor and other suspicious lymph nodes at the level of the submandibular and hyoid bone in the absence of distant metastases, with a staging of T4, N2, M0, and a G1 grade carcinoma at the pathological exam.

Case 2 was an 83 years old woman with an adenocarcinoma (G2) of the left submandibular gland with mandibular bone involvement and spontaneous bleeding, and without distant metastases who was staged as T4 N2 M0.

Case 3 was a 53 years old man with a recurrent lower lip carcinoma and submandibular lymph nodes involvement. He was operated for the first time in Bulgaria for tumor resection without laterocervical neck dissection. Some months later he was operated in Italy two more times for recurrence of the tumor and then he was referred to our Department where he was staged as an inoperable T4 N2 M0 because of carotid infiltration at CT scan