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Mini Communication

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# In vitro Profiling of Indian Patients Head & Neck Tumor Derived Spheres

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Head and Neck (H&N) cancers are malignant types contributing to one-third of all cancer types in India [1]. The actual burden of this cancer type in India is much greater than reported and reflected through the existing literature. South-East Asia is documented to face steep increase of over 75% in the number of cancer deaths in 2020 as compared to 2000. Also, on the logic of Indian population becoming nearly twice that of the world in the past 15 years, increase in cancer burden with the same proportion is anticipated. Like other tumor types, H&N tumor also resides heterogeneous cell populations and is diverse both at molecular and cellular levels. Among the diverse primary cell types in the tumor microenvironment, the presence of tumor causing Cancer Stem Cells (CSCs) was reported in the invasive fronts of the H&N tumor, near blood vessels [2]. Markers such as Aldehyde Dehydrogenase (ALDH), CD133, and CD44 have been reported on highly tumorigenic CSCs segregated in the primary cultures [3]. These CSCs are shown to be associated with resistance to therapies, metastasis, relapse and reactivation of malignancy in the patients. Like therapeutic stem cells, CSCs augment their progeny by forming special colonies from single tumor cells. These special colonies or spheres are sometimes referred as tumorospheres that are further sorted into three phenotype varieties: holospheres, paraspheres, merospheres [4]. Degree of clonogenic potential, heterogeneity within these spheres were speculated to be influencing tumor progression, metastasis and refraction to chemo drugs along with the spectrum of critically important related factors, although race and ethnicity correlation was never made in the literature. In this mini communication, we report the classification of our observation of the invitro experimental data obtained from 3 different H&N cancer patients from India with regards to the orosphere/tumorosphere subtype and occurrence.

With Informed consent of patients and the Ethics Committee approval, post-surgical specimens were collected from three (F, 72yr; M, 61yr; F, 40 yr) Head and Neck cancer patients and transported immediately from the operation theater to the laboratory for processing. Indian Head and Neck Cancer Patient derived Malignant Primary (IHN-PMP) Cell lines were harvested as published [4].

We found that all the three IHN-PMP Cell lines growing in ultra-low adhesion conditions generated three morphologically distinct sphere populations namely: holospheres, merospheres, and paraspheres (Figure 1A-D). PM Cell lines-IHN generated a higher (3X) number of holospheres compared to merospheres (1.5X) paraspheres (2X).

Three tumorospheres from holospheres, merospheres and paraspheres types were isolated by pipetting and seeded independently onto tissue culture coated dishes that allowed cell attachment. Spheres were followed for 7 days, and the number that attached was quantified. Holospheres were found to be adhering better to the dish surface than merospheres and paraspheres. All holospheres adhered to the substrate within the first nine hours of seeding with cells spread out by day three, while merospheres and paraspheres adhered after 24 hr of seeding.

All the three tumorospheres types when were isolated by morphology, dissociated into single cells to seed with uniform inoculating density of 20,000 cells onto ultra-low adhesion wells, the distinct observation

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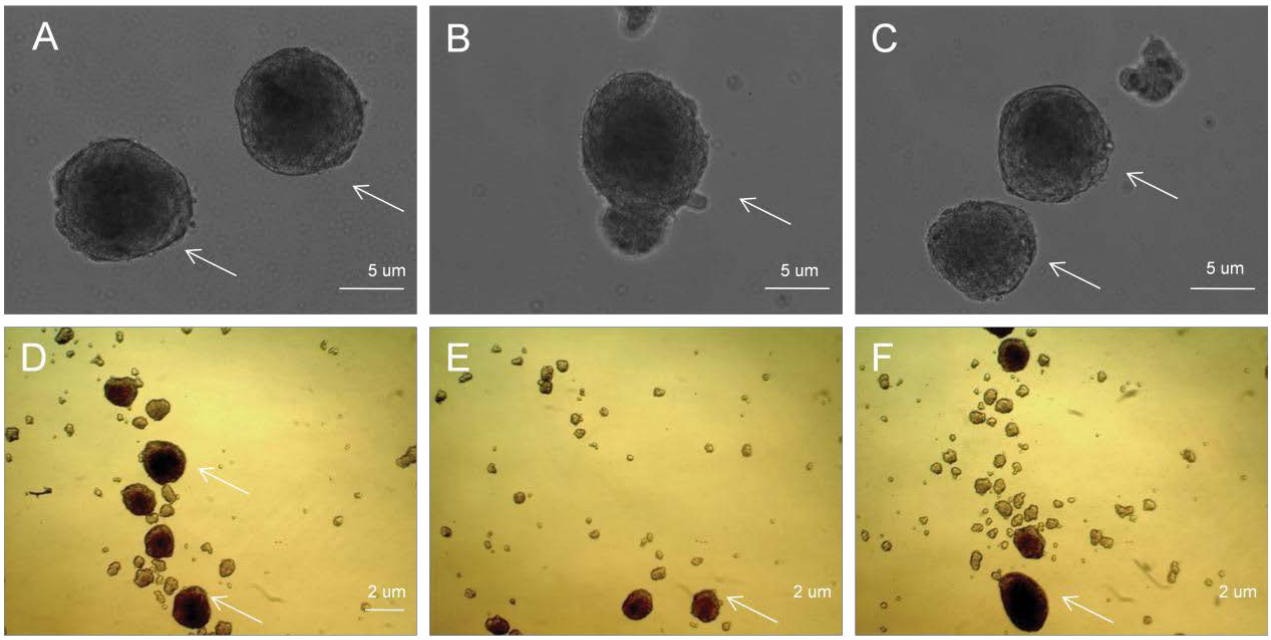
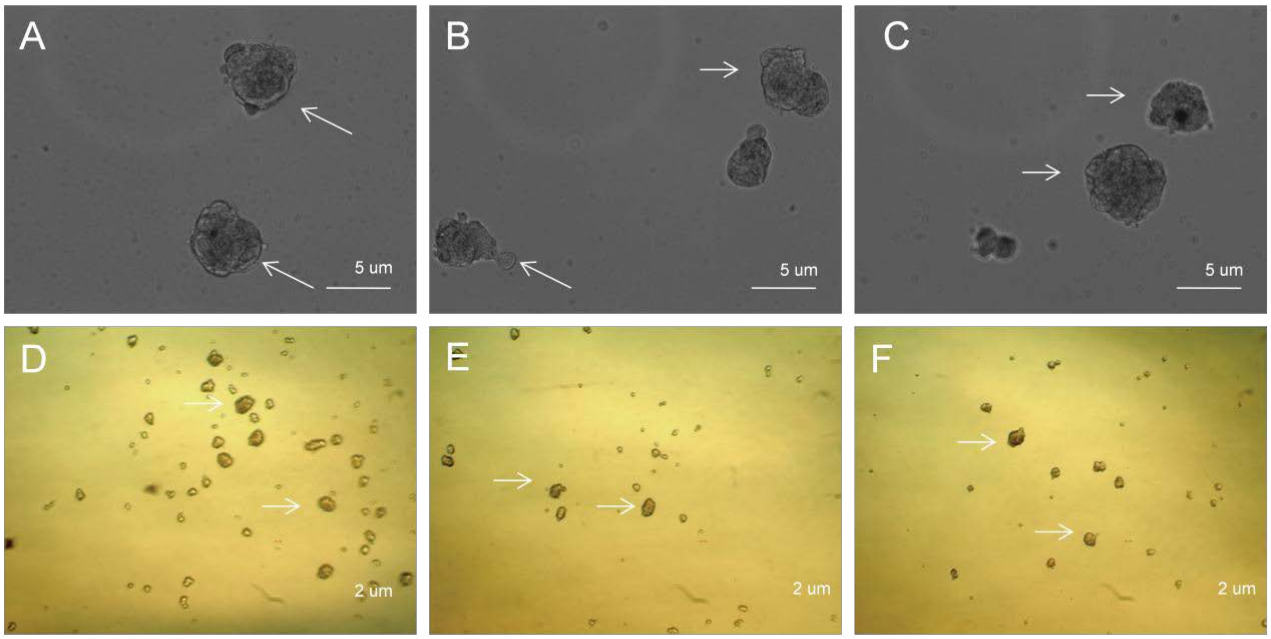



A, B, & C Holospheres captured in Floid Imaging Station at 20x Magnification.
D, E & F Holospheres captured by Phase contrast microscope at 10x Magnification

**Figure 1A**

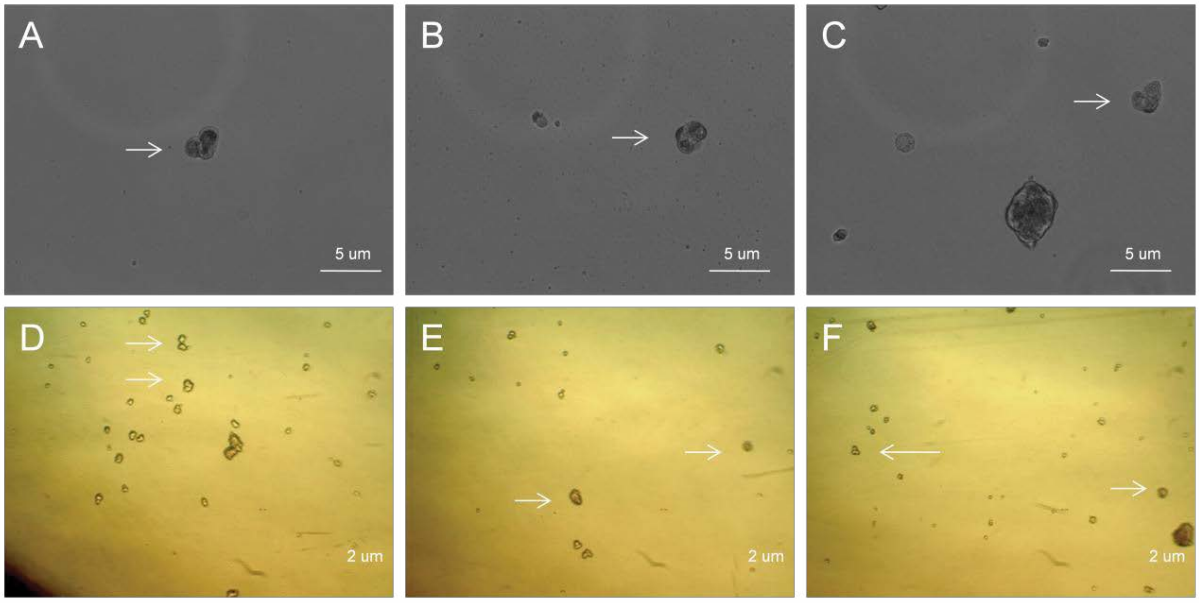



A, B, & C Merospheres captured in Floid Imaging Station at 20x Magnification.
D, E & F Merospheres captured in phase contrast microscope at 10x Magnification

**Figure 1B**

after three days of: all the three types of tumorospheres only in the wells with single cells of holospheres seeded was made. Also, the ratio of holospheres, merospheres and paraspheres reformed in the wells seeded with single cells of holospheres was 5:2:1.

While we document signature culture characteristics of holospheres derived from Indian H&N cancer patients (only from three) sourced tumor biopsies, we speculate the connection with CSCs residing in holospheres irrespective of the ethnic and clinical features of the source material. However, our data does not signify the statistics of observed features. There are aggressive malignancy types of H&N cancer manifestations with high mortality rate from Indian population as reported in 2014 [5]. Major challenges evidenced for this disease management include development of resistance to the traditional chemo therapy and early formation of metastases. CSCs have emerged as important tools in both pathologic mechanisms and treatments. Therefore, targeting holospheres for genomics typing to search for new biomarkers of cancer stem cells for Indian patients in diagnosing the disease along with screening for drugs on these holospheres that kill them would be an ideal




A, B, & C Paraspheres captured in Floid Imaging Station at 20x Magnification.
D, E & F Paraspheres captured in phase contrast microscope at 10x Magnification

**Figure 1C**

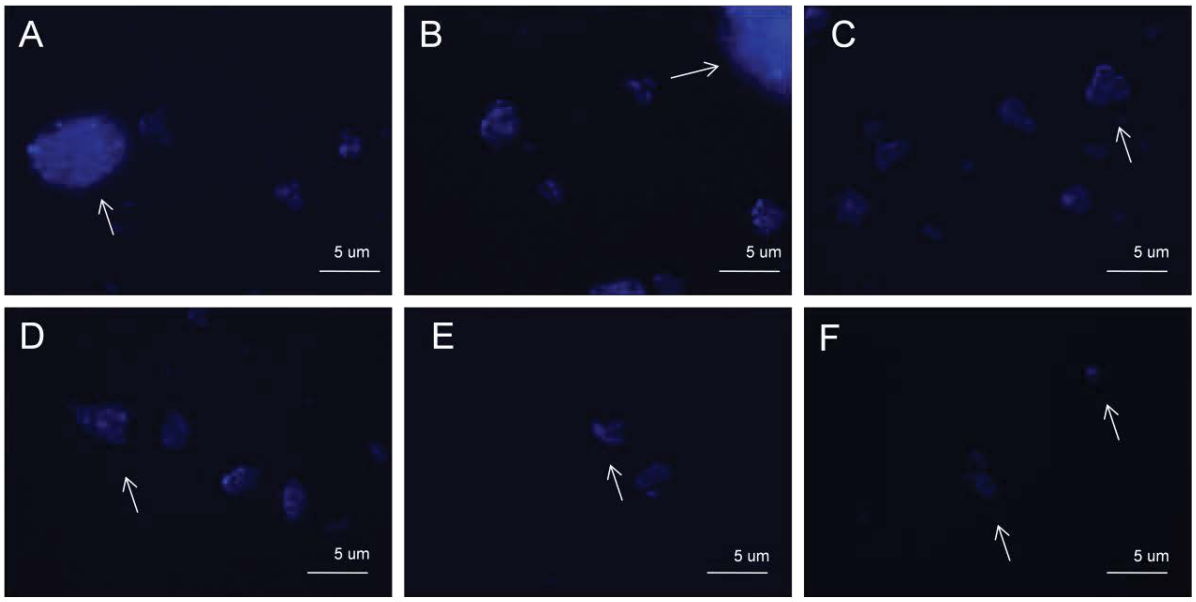



Image A & B showing Stained Holosphere in Floid Imaging Station at 20x Magnification
Image C & D showing Stained Merosphere in Floid Imaging Station at 20x Magnification
Image E showing Stained Paraspheres in Floid Imaging Station at 20x Magnification
Image F showing Stained single cells in Floid Imaging Station at 20x Magnification

**Figure 1D**

strategy of drug discovery and development in Oncology. Personalized therapy based on molecular analysis and pharmaco-genomic studies on these patient derived cultured samples could be the way forward to invest the scientific resources focusing on Indian specific variations for better understanding the drug responses and adaptation as well.

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