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Research Article

Differential expression of mitotic regulators and tumor microenvironment influences the regional growth pattern of solid sarcoma along the cranio-caudal axis

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ABSTRACT

Soft tissue sarcomas are relatively rare, unusual, anatomically diverse group of malignancies. According to the recent literature and medical bulletins, tumor growth and aggressiveness immensely relies on its anatomical locations. However, it is unclear whether the cranio-caudal anatomical axis of the mammalian body can influence sarcoma development and the underlying molecular mechanisms are not yet deciphered. Here, we investigated the growth pattern of solid sarcoma implanted into the murine cranial and caudal anatomical locations and tried to explore the location specific expression pattern of crucial mammalian mitotic regulators such as Aurora kinase A, Histone H3 and c-Myc in the cranio-caudally originated solid tumors. In addition, the influence of local tumor microenvironment on regional sarcoma growth was also taken into consideration. We found that solid sarcoma developed differentially when implanted into two different anatomical locations and most notably, enhanced tumor growth was observed in case of cranially implanted sarcoma than the caudal sarcoma. Interestingly, Aurora kinase A and c-Myc expression and histone H3 phosphorylation level were comparatively higher in the cranial tumor than the caudal. In addition, variation of tumor stroma in a location specific manner also facilitated tumor growth. Cranial sarcoma microenvironment was well vascularized than the caudal one and consequently, a significantly higher microvessel density count was observed which was parallel with low hypoxic response with sign of local tumor inflammation in this region. Taken together, our findings suggest that differential gradient of mitotic regulators together with varied angiogenic response and local tumor microenvironment largely controls solid sarcoma growth along the cranio-caudal anatomical axis.

1. Introduction

Non-epithelial, extra-skeletal tissues of the mammalian body are collectively known as soft tissues which include muscles, fibrous tissues, cartilage, blood vessels, etc and are the primary sites of the evolution of Soft tissue solid sarcoma (STS). STS can be categorized as a heterogeneous group of malignant neoplasms of mesenchymal origin and is the most prevalent type of childhood cancer. It comprises 7.4% of all cancer cases in this age group. According to the reports of the National Cancer Institute, the annual incidence of STS in USA is approximately 11,660 cases and overall mortality rate is nearly 3820 cases per year [1–5]. Inherited genetic abnormalities and chromosomal mutations are considered widely as possible causes of sarcoma development. Our previous investigation revealed that local tumor microenvironment, including various cadherin molecules and cytoskeletal filaments also influences different forms of sarcoma development (solid and ascitic) [6]. However, the influence of the cranio-caudal anatomical axis of the mammalian body on sarcoma development and its underlying molecular mechanisms are largely unknown. Soft tissue sarcomas are anatomically diverse and it has been documented in many literatures that tumor growth and aggressiveness relies on its anatomical locations. As, for example, a study performed in mice revealed that administration of a carcinogen into two different anatomical sites i.e in the inter-scapular and sacral region, resulted in accelerated tumor growth and malignant progression in the inter-scapular region as compared to the sacral region [7]. The findings were later explained by the pioneering work of Kobayashi who reported about the regional difference in epidermal mitotic activity adjacent to a wound in response to skin injury in mice. The study demonstrated that high mitotic activity was displayed by the cells located in the anterior parts of the body,

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whereas mitotic activity gradually decreased from middle to posterior parts of the body. The study suggested the existence of a cranio-caudal mitotic gradient in the epidermis of mice in response to injury [8]. Another two contemporary articles showed the phenomenon of regional differences in tumor growth following intradermal, subcutaneous and intraperitoneal tumor implantation into two different anatomical locations of the mice. They observed rapid tumor growth in the anterior parts of the body as compared to the posterior parts [9,10]. The group hinted about the regional differences in the vascular system, possibly controlling tumor growth in the various anatomical locations, but they were unable to provide enough satisfactory explanations behind this event [11]. Further investigations showed that tumor growth near the scapulae was much faster than the same tumor growth near the iliac crest following subcutaneous tumor implantation. The mechanism controlling regional tumor growth was not identified. A recent study revealed that tumor growth in the upper abdomen was much faster than the lower abdomen following implantation of CT26 colon carcinoma cells in the murine peritoneal cavity. Differential angiogenic response between the upper and lower abdomen due to the gradient of thrombospondin-1 (TSP-1), an endogenous angiogenesis inhibitor expressed in the tumor microenvironment, regulating differential tumor growth in this case [12]. Till date, many hypotheses have been proposed to explain the regional control of tumor growth phenotype and behavior, but only few of them explained the event satisfactorily. However, no attempt has been made to evaluate the role of different crucial mitotic regulators in the event of differential control of tumor growth.

The mammalian cell cycle is an extremely organized and tightly regulated physiological process, the deregulation of which may eventually lead to the evolution of cancer [13]. Chromosome remodeling plays a pivotal role during cell division,which is carried out by multiple post translational modifications of core histone proteins (H2A, H2B, H3, and H4) of the nucleosome complex such as phosphorylation, acetylation, methylation etc. Particularly, phosphorylation of Histone H3 at conserved serine 10 residue is involved in the transcriptional activation as well as in chromosome condensation during mitosis and meiosis in various organisms [14,15]. In mammalian cells, histone H3 phosphorylation at Ser10 is a dynamic process, begins in the late G2 phase, maintains the phosphorylated form during metaphase and then, gradually dephosphyralates at late mitotic phases [14]. Increased phosphyralation of histone H3 at Ser10 has implicated in neoplastic transformation and carcinogenesis [16–18]. Histone H3 phosphyralation is mediated by the members of the aurora kinase family, an evolutionarily conserved serine–threonine mitotic kinase, which is known to regulate processes like centrosome duplication, spindle formation, G2/M check point control during cell division [19,22]. Among the three identified mammalian Aurora homologs (A, B, C), Aurora-A and Aurora-B are known to be capable of phosphorylating histone H3 at ser10 during G2/M transition. Furthermore, Aurora-A has been considered as a better histone kinase and frequent amplification of its chromosomal location (20q13) has been documented in many cases of human malignancies [19–22]. Elevated level of P-histone H3 (Ser10) has been found in many human cancer cases, which is correlated with overexpression of aurora kinase family members [23] [24]. Moreover, chromosomal instability and malignant transformation have been found in various in vivo and in vitro studies which is correlated with high aurora kinase expression level and increased mitotic H3 phosphorylation (Ser10) [25]. Over expression of both Aurora A and B has been found in Ewing sarcoma-derived cell lines where targeted therapy against Aurora A and B has shown anti-tumor activity [26,27]. Over-expression of Aurora kinase A gene has also been detected in rhabdomyosarcoma tumors [28]. Moreover, inhibition of Aurora kinase down regulates P-Histone H3 expression,which is accompanied with suppression of synovial sarcoma growth and malignant progression [29]. Interestingly, c-Myc oncoprotein regulates Aurora kinase A expression and subsequent mitotic progression and moreover, by a feedback loop c-Myc expression is also controlled by Aurora kinase-A. Elevated c-Myc level has been found in many STS cases with poor survival rate [30–32].

In addition to the mitotic regulators, the interaction of neoplastic cells with evolving local tumor microenvironment is also indispensable for solid sarcoma growth [6,33]. Microvessels are important microenvironmental components of the cancer tissue and are required to provide oxygen and nutrients to the growing neoplastic cells. Rapid tumor growth is associated with alterations in microenvironmental oxygen tension and often causes tissue hypoxia (oxygen deprived condition), due to lack of adequate vasculature or structural abnormalities of the existing blood vessels failing to supply enough oxygen to the rapidly dividing cancer cells. Prolonged hypoxia can severely restrict tumor growth [12] as well as can have a negative impact on malignant cell survival [34]. Thus, mammalian system has evolved HIF-1α aunique sensor to maintain tissue oxygen homeostasis. The transcriptional activation of HIF-1α is an immediate response to tissue hypoxiaand it is a hypoxically responsive component of the Hypoxia-inducible transcription factor (HIF) complex [35–36]. Moreover, HIF-1α has been widely accepted as a marker to measure the level of O_2 tension in cancer tissues [37]. Apart from the endothelial capillary networks, tumor stromal components like tumor associated fibroblasts (CAFs) and adipocytes (CAAs), inflammatory cells, etc. are also involved in the modulation of extracellular tumor matrix and generation of inflammatory response through secreting plethora of pro-tumorigenic chemokines ultimately facilitating tumor growth, invasion and metastasis [33,38].

In the present study, we mainly put emphasis on the following points: (i) whether there is any differential growth pattern of solid sarcoma along the cranio-caudal anatomical axis, (ii) expressional alterations of mitotic regulators, mainly Aurora kinase A, c-Myc and Histone H3 and (iii) influence of local tumor microenvironment behind the regional growth pattern of solid sarcoma along the above anatomical axis.

2. Materials and methods

2.1. Animal maintenance

Inbred Swiss Albino mice of both sexes (age, 10–12 weeks; weight, 20–22 g) were obtained from the institutional animal colonies, Calcutta School of Tropical Medicine, Kolkata, India. Animals were provided proper diet, water ad libitum and sterile paddy husk as bedding throughout the experiment. Standard temperature (22 ± 2 °C), humidity and 12 h dark-night cycle was maintained. All animal protocols regarding animal maintenance and experiments were abide by the guidelines of the Institutional Animal Ethical Committee (IAEC) and EU Directive 2010/63/EU..

2.2. Disease induction

Cockers's sarcoma (ascitic Sarcoma-180) was used for the purpose of experimental sarcoma development in mice. Briefly, malignant ascitic fluid was aspirated from the peritoneal cavity of tumor bearing animal by using an 18-gauge needle and sterile syringe. 100 μl of the fluid was sent for microbial contamination assessment and a small volume of the fluid was tested for cell viability estimation using trypan-blue dye exclusion method. The ascitic fluid was then diluted into sterile isotonic PBS solution