Biol Syst Open Access 2015, 4:2
http://dx.doi.org/10.4172/2329-6577.S1.003

OMICS International
conferenceseries.com

3rd International Conference on
Integrative Biology

August 04-06, 2015 Valencia, Spain

Drosophila muscle specification in the reproductive system: A model for hormonal disruptors testing

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The specification of different structures in animals is frequently dependent on signal coming from nearby tissues. We have addressed this problem by studying the determination of reproductive system muscles that surround the male testes. We have found that the correct development of these muscles which are smooth ones requires the activity of the genes Six4, Abdominal-B and Drop in the muscle cells. To study if there are signals that may also affect muscle development, we decided to explore the tissue that surrounds this muscle formed by cells known as pigment cells. These are a male specific type of cells derived from the fat body which surrounds the gonads and give the specific yellow color to the testes. We have studied the role of these cells during muscle cell fate determination and have observed that pigment cell alteration triggers muscle defects. One modification that entails dramatic aberrations in muscles and testes is the sex reversal of these cells that is changing them to female cells. Therefore, this muscle is very sensitive to sex alterations. There is growing concern that accumulation of contaminants may produce serious change in animal species including disruption of hormones that contribute to sex determination. We propose this *Drosophila* tissue as a good candidate to study the genetic alterations due to hormonal disruptors and other contaminants.

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Evaluation of the interaction of copper (II) and ruthenium (II) compounds with fibronectin and tubulin proteins, two potential chemotherapeutic targets

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Most current research efforts with respect to the quest for novel metal-based drugs are devoted to investigating their interaction with the DNA double helix considering the accepted mechanism of action for the well-established anti cancer drug cisplatin. However, this approach neglects the fact that other cellular components may be targeted by metal complexes. In the present study, fibronectin and tubulin, two proteins involved in fundamental cellular processes that are cell division and cell migration have been chosen as (cytotoxic) targets for copper (II) and a ruthenium (II) compounds. The potential interaction of a series of metal-based molecules with these two proteins was first assessed by circular dichroism (CD) and atomic-force microscopy (AFM). MTT assays were subsequently used to determine the cell-growth inhibitory activities (IC50) of the compounds in HeLa and HL60 cell lines. Immunofluorescence assays were then carried out with the two most cytotoxic metal complexes with HeLa cells using anti-α, anti-β-tubulin and anti-fibronectin antibodies to investigate their effect on microtubules and the extracellular matrix. The microtubule-depolymerizing agent Nocodazole was used as positive control. Cell cycle analyses by flow cytometry and annexin V-FITC+PI apoptosis assays (with cisplatin as positive control) were performed with both cell lines to better understand the mechanisms of action of the two compounds. The AFM and CD experiments clearly evidenced the interaction of the Cu (II) and Ru (II) compounds with both proteins, the most efficient being the copper molecule. IC50 values lower than those of the reference compound cisplatin were obtained for both the Ru (II) (13.01 μM HeLa; 2.48 μM HL60) and the Cu (II) (3.63 μM HeLa; 14.50 μM HL60) complexes. The immunofluorescence assays revealed a microtubule-depolymerizing behavior for the copper molecule and the formation of apoptotic nuclei with both compounds. The cell cycle tests did not show an arrest at the G2 phase which would have indicated microtubule stabilization (that prevents cell division), therefore suggesting that the cells had died by apoptosis as confirmed by the annexin assays. In summary, fibronectin and tubulin are legitimate target proteins for potential metal-based anticancer drugs such as the two compounds evaluated herein which showed apoptotic cytotoxic activities.

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