TINJAUAN: POTENSI EPHA2, EPHA3 DAN EPHRIN-A1 SEBAGAI TARGET TERAPI OSTEOSARKOMA PADA ANJING

Laut M1, Palmieri C2, Allavena R2

1Bagian Farmakologi, Fakultas Kedokteran Hewan, Universitas Nusa Cendana, Kupang, Indonesia; Email: meity_marviana@yahoo.com
2Jurusan Kedokteran Hewan, Fakultas Sains, Universitas Queensland, Brisbane, Queensland, Australia

INTISARI

Osteosarkoma pada anjing (OSA) adalah jenis kanker tulang yang paling penting dianggap pada anjing dengan karakteristik utamanya adalah derajat keganasan yang tinggi dan mudah menyebar, perkembangan yang sangat cepat, prognosis yang jelek dan harapan hidup pasien yang pendek bila metastasis terdeteksi. Terapi osteosarcoma yang ada sekarang tidak dapat meningkatkan harapan hidup pasien. Oleh karena itu, sangat penting untuk meneliti agen terapi OSA yang baru. Reseptor ephrin (Ephs) merupakan kelompok reseptor utama dari keluarga Tirosin Kinase (RTKs) dan berfungsi penting dalam perkembangan tumor dan proses metastasis. Adanya overekspresi EphA2, EphA3 dan protein ligannya (Ephrin-A1) pada berbagai tumor pada manusia, mengindikasikan keganasan tumor, prognosis yang jelek dan harapan hidup pasien yang singkat. Tujuan utama tinjauan ini adalah untuk mengetahui overekspresi mollekul-molekul tersebut diatas dan fungsinya pada berbagai tipe kanker pada manusia maupun hewan. Hasil tersebut diharapkan dapat menjadi dasar untuk pengembangan sistem diagnosta dini OSA dan penentuan terapi yang efektif yang dapat berkontribusi langsung terhadap peningkatan harapan hidup pasien.

Kata kunci: EphA2, EphA3, Ephrin-A1, osteosarkoma anjing, overekspresi

Canine Osteosarcoma

The high grade malignant tumors arising from bone, osteosarcoma (OSA), appears more frequent than other bone cancer in dogs and leads to death within several months prior to diagnosis. This bone cancer in dogs is characterized by highly aggressive, rapid progression, early metastases primarily to lungs, and infrequently metastases to regional lymph nodes, resulting in poor prognosis and short life expectancy (Misdorp & Hart 1979; Dernell et al. 2001). The precise etiology of canine OSA has not been defined yet, but several risk factors are found related to the development, as reviewed below.
Firstly, OSA is a breed-related disease. Past publications reported the occurrences of OSA in the Great Dane, Irish setter, St. Bernard, Rottweiler, Boxer and German shepherd (Liu et al. 1977; Brodey 1979). Secondly, the range of age (young (1 year) to old (15 years) with 7 years being an average age), will play a role, with age progression posing a greater risk to developing this disease (Ru et al. 1998). OSA incidences in large and giant breed dogs mostly develop in early age (Misdorp & Hart 1979; Mauldin et al. 1988). The reason was the growth of the epiphysis terminates around 11 months. Thus, in more mature dogs, the cartilaginous growth plate is replaced by bone (Brodey 1979).

Another factor related to canine OSA development is the most common affected sites. Earlier studies reported humerus, radius-ulna, femur and tibia in the appendicular skeleton and the flat bones in the axial skeleton as the most common sites of dog OSA. The main reason is the long bones function in supporting the body weight. The same reason explained why OSA incidences were predominant in the front limb rather than the hind limb. OSA primary neoplasm was mostly present on the metaphysis and/or epiphysis of the bones, whether arising in the distal or proximal part. The suspected areas of OSA lesions are in the proximal part of humerus and tibia, together with the distal part of radius and femur (Dernell et al. 2001; Endicott 2003; Mueller et al. 2007).

Axial OSA occurs in other bones apart from limbs, with maxilla and mandible are the common affected bones (Liu et al. 1977; Mueller et al. 2007). This type is not associated with large and giant breeds and not characterized by rapid tumor growth, except for cancer originating from the ribs which were reported to be aggressive than other axial osteosarcomas. Lastly, extra skeletal OSA, which originate from other parts of the body (such as the mammary gland, spleen, kidney, liver, testicle) were reported as very rare occurrences in dogs. However, the prognosis of these incidences is worse than the appendicular OSA as it is difficult to remove the primary tumor from the affected sites (Dernell et al. 2001).

Dogs with appendicular OSA appeared very lame with or without signs of pain, and struggling from swelling on the predilection sites as a result of tumor extension into adjacent tissues (Brodey 1979; Mueller et al. 2007; Morello et al. 2011). To define a final diagnosis of OSA, a combination of clinical appearance, complete history and the radiography images are essential and helpful to support the microscopic findings, to predict the malignancy of the cancer, the possibility of metastasis and the application of the correct therapy. Diagnosis of OSA should be made through biopsy from the site of tumor origin, histologic diagnosis through primary tumor samples and lymph nodes submitted (Mueller et al. 2007; Morello et al. 2011).

Treatment options for OSA range from surgery, radiotherapy, chemotherapy, and combination of chemotherapy and adjuvant agents and non-curable therapy to manage pain in patients (Endicott 2003; Morello et
Although, the existing therapy could help to improve patient survival time, none can be suggested as successful and satisfied treatment. Amputation was shown to enhance life-span and provide a pain free life in affected dogs. However, metastases develop months after surgery and result in a very poor prognosis (Chun & de Lorimier 2003). Moreover, a lack of successful chemotherapeutic agents and prohibitive cost of treatment have also become limitations in canine OSA therapy scheme (Mueller et al. 2007). In short, it can be said that the improvement in treatment strategies were not followed by improvement in patient survival time once metastases is detected.

**Classification**

In diagnosing canine OSA, the radiographic features, apparent clinical signs, and history, are valueless without microscopic evaluation. Thus, an international agreement for domestic animal tumors was established and determine the histological features of canine OSA. There are six histosubtypes of canine OSA: osteoblastic, fibroblastic, chondroblastic, telangiectatic, poorly differentiated and giant cell type. Canine OSA is considered heterogeneous as it shows variation in morphologic and neoplastic cells arrangement. Thus, histological patterns of canine OSA was made based on the type and amount of dominant matrix intercellular, and forms of neoplastic cells (Slayter et al. 1994).

Osteoblastic is classified into productive type in which abundant foci of osteoid or woven bone identified near the blood vessels and the non-productive type in which minimal osteoid is produced. Osteoids are arranged haphazardly and randomly in these type. Fibroblastic subtype characterized by prominent malignant spindle-shaped cells. Chondroblastic type is identified through the presence of islands of cartilage. Telangiectatic is characterized by the presence of numerous and large cysts filled with blood and covered by a layer of malignant cells, which replaced the original bone structure. Giant cell type is identified through number of neoplastic cells which are fused together and form large giant cells. The same tumor may show a combination of these subtypes (Nagamine et al. 2015).

**Ephrin Receptors**

Ephrin receptors (Ephs) represent the main group of receptors of the tyrosine kinases (RTKs) family, a transmembrane protein with capability in recognize intracellular responses. Ephs have essential cellular functions: control the interaction between cells and migration of cells. Ephs also play an important role in tumor development and metastases process. Basically, the Eph family is classified into two subtypes, type A and type B. Each type is activated by a group of transmembrane proteins known as ephrins. Eph type A consists of eight members (EphA1-A8), activated by five members of
Ephrins (EphrinsA1-A5), while Eph type B consists of six members (EphB1-B6) and activated by the three ephrin B members (B1-B3) (Kandouz 2012; Barquilla & Pasquale 2015).

Generally, the expression of Eph receptors and their Ephrin ligands, observed in high levels during embryogenesis, and reduced levels in adulthood (Wykosky & Debinski 2008). In relation to tumor development, the receptors may support tumor growth, inhibit, or both. Moreover, Eph receptors play an important role in the formation of new blood vessels, which are essential in metastases process. All of these functions have been investigated through studies of various human cancers and concluded that the receptors overexpression associated with malignancy and poor prognosis. Therefore, it was suggested that each type of ephrin receptors and their ligands may serve as potential diagnostic tool and therapeutic target in cancer treatment, which is proved to be helpful in predicting disease development in the early stages and may affect patient survival rates (Lackmann 2010; Xi et al. 2012b; Barquilla & Pasquale 2015).

Receptor EphA2

EphA2 is the most widely studied and investigated in various types of human cancers (Thaker et al. 2004; Holm et al. 2008). Although, it mostly discovers in epithelial tissues, the expression is not limited on the particular tissues. The receptor present in the developing bones, lung, and thymus. Meanwhile, an elevated levels of EphA2 present in lungs, skin, small intestines, and ovaries, while reduced levels discover in brain and spleen. However, the receptor was not detected in the heart, liver, and testes in adults (Zhou 1998).

In relation to cancers, EphA2 expression in tumors derives from the demonstration of its relationship with the tumor grade and aggressiveness when overexpressed. Hence, it may be useful to define the staging and prognosis of each particular tumor (Tandon et al. 2011). EphA2 overexpression is found in squamous-cell carcinoma of the head and neck (Liu et al. 2011), pulmonary cancer (Li & Xie 2013), mammary gland cancer (Vaught et al. 2008), skin cancer and cancer of brain and spinal cord (Tandon et al. 2011), prostate cancer (Xi et al. 2012b; Lisle et al. 2013), ovarian cancer (Thaker et al. 2004), and adenocarcinoma of pancreas (Giaginis et al. 2010). The expression was observed in patients with advanced stage of cancers and associated with poor prognosis, while patients with low-levels of EphA2 showed better prognosis (Tandon et al. 2011; Li & Xie 2013). Similarly, EphA2 overexpression was observed in ovarian carcinoma, while no or low-level of EphA2 present in benign type and the non-neoplastic ovaries. The result confirmed the hypothesis that up-level of EphA2 is associated with tumor aggressiveness (high grade tumor) and short survival time. Thus, it is suggested that EphA2 in ovarian carcinoma is a potential marker of cancer malignancy and has potential as therapeutic target (Thaker et al. 2004).
addition, Liu et al. (2011) reported similar result in human esophageal carcinoma, in which significant level of EphA2 was expressed in half specimens. Furthermore, the receptor also expressed a significant level in renal cancer cells and confirmed the correlation with more aggressive cancer (Thaker et al. 2004). Lastly, a recent study by Fritsche-Guenther et al. (2010), observed the overexpression of EphA2 in human osteosarcoma samples and no expression detected in normal bone. An elevated level of EphA2 in this study has suggested EphA2 as a potential therapeutic target in osteosarcoma.

**Receptor EphA3**

Another important receptor of the Ephs type A subfamily is EphA3, which reported to be expressed during neovascularisation and metastases process. Tumor angiogenesis or forming of new vasculature is a significant sign of metastases development which is characteristic of highly aggressive tumor (high grade). (Surawska et al. 2004). Normally, in adult human, the receptor is identified mainly in the brain, while in embryo development, kidney, lung, heart, and muscles in the axial skeleton, thymus, and liver showed insignificant level. The skeletal muscles and bones expressed up-levels of the receptor (Zhou 1998).

In relation to tumorigenesis, overexpression of EphA3 was reported in human colorectal cancer, in which the immunostained positive cells were recorded in the cytoplasm of neoplastic cells. It was concluded that the overexpression associated with poor life expectancy (Xi et al. 2012a). The similar finding was reported from a study (Lu et al. 2013) in liver carcinoma, in which up-levels of EphA3 was recorded and shown correlation between cancer progression and survival time. Moreover, EphA3 was reported to be associated with B and T cell cancers (Xi & Zhao 2011). In addition, another study (Xi et al. 2012a) confirmed high level of EphA3 in gastric carcinoma and low-level in normal tissue. This finding also associated with tumor staging and poor prognosis. Overall, EphA3 can be suggested as a useful target for therapeutic and a potential indicator of tumor prognosis.

**Receptor Ephrin-A1**

Ephrin-A1 is the primary ligand of receptor EphA2. Thus, the interaction of EphA2 and ephrin-A1, created a specific characteristic of two-directions molecular signaling, which is essential in cellular response. The signal is a forward signaling from the Eph receptors and a reverse signaling from the ephrin ligands (Surawska et al. 2004; Liu et al. 2011; Ieguchi et al. 2013). The interaction of EphA2 and Ephrin-A1 initiates tumor growth and supports its development. Thus, a high level of EphA2 and Ephrin-A1 indicates the severity and prognosis. This interaction has been identified in skin cancers, where Ephrin-A1 has an important role as melanoma growth factor (Giaginis et al. 2010; Beauchamp & Debinski 2012).
A study in investigating ephrin ligands expression in human osteosarcoma was conducted by Varelias et al. (2002), in which expression of Ephrin-A1 was recorded in majority of samples. The result suggested that osteoblasts may present Ephrin-A1. Furthermore, a study by Fritsche-Guenther et al. (2010) observed insignificant levels of Ephrin-A1 in adult normal bone tissue and high-levels in OSA samples. The result suggested possible role of Ephrin-A1 and interaction with EphA2 as potential therapeutic target in OSA. Moreover, Giaginis et al. (2010) was reported significant staining of Ephrin-A1 in pancreatic ductal carcinoma and suggested that the expression has a correlation with tumor volume and histopathological stages. Similarly, previous studies in colorectal cancer and hepatocellular carcinoma reported significant levels of Ehrin-A1 and suggested its association with with poor prognostic (Ieguchi et al. 2013).

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